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INSECT ALLERGY AS A POSSIBLE CAUSE OF INHALANT SENSITIVITY

RICHARD D. WISEMAN, M.D., WILLIAM G. WOODIN, M.D.,
HOWARD C. MILLER, PH.D., AND MORTON A. MYERS,* SYRACUSE, N. Y.

DURING the past two years our group has been actively studying insect allergy and its possible relationship to inhalant-respiratory-allergic disease. It has been our purpose to make this work a pilot study in an attempt to cover broad areas rather than to confine our work to certain groups of insects or stages of insect life. We therefore prepared our extracts from ten of the twenty-eight commonly recognized different orders of the class *Insecta*. Thirty-three different extracts were made from twenty-seven species of insects that were either reared or collected in pure samples by competent entomologists. Extracts were prepared by the standard technique, which included dialysis, and then were standardized by means of protein nitrogen units for skin-testing purposes.

Antigens were made from both the larva and the adult of several species, and the pupa and the adult of one species. We prepared our material in this manner with two thoughts in mind: (1) to learn whether common antigens were present in different orders of the class *Insecta* as well as among members of the same order and (2) to determine if there were common antigens present in the various stages of the life cycle of the same insect. Table I shows the insects studied.

Complete metamorphosis takes place in most orders of the class *Insecta*. This type of life cycle is demonstrated in Fig. 1, which shows how the tent caterpillar goes through the egg stage, which is followed by the larva and the cocoon (which contains the pupa) stages, and later becomes the adult moth in the final stage of the metamorphosis.

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The pupa of this and many other species is enclosed within a silken covering known as a cocoon. This type of cocoon, however, is not necessarily characteristic of all moths. Fig. 2 demonstrates the other type of metamorphosis, namely, the incomplete type, which is quite common to many insects. In incomplete metamorphosis the insect emerges from the egg with the same body structure that is later seen in its adult life, and during metamorphosis it merely enlarges and finally matures. This is most clearly represented by what occurs in the order *Orthoptera,* which includes the grasshopper, the insect whose life cycle is pictured in Fig. 2.

TABLE I

COMMON NAME	SCIENTIFIC NAME OF SPECIES	ORDER
1. Field cricket (adults)	*Acheta assimilis F.*	Orthoptera
2. American cockroach (adults)	*Periplaneta americana L.*	Orthoptera
3. Fish fly (adults)	*Chauliodes pectinicornis L.*	Neuroptera
4. May fly (naiads)	*Hexagenia limbata Say.*	Ephemerida
5. Damsel fly (adults)	*Agrion maculatum Beauv.*	Odonata
6. Dragonfly (adults)	*Plathemis lydia Dry.*	Odonata
7. Meadow spittle bug (adults)	*Philaenus leucophthalmus (L)*	Homoptera
8. Goldenglow aphid (adults)	*Macrosiphon rudbeckiae (Fitch)*	Homoptera
9. Green peach aphid (adults)	*Myzus persicae (Sulz.)*	Homoptera
10. Meadow plant bug (adults)	*Miris dolobratus (L)*	Hemiptera
11. Carpet beetle (larvae) (cast skins)	*Trogoderma versicolor Creutz*	Coleoptera
12. Japanese beetle (adults)	*Popillia japonica Newm.*	Coleoptera
13. European chafer (adults)	*Amphimalon majalis (Razoum.)*	Coleoptera
14. Red milkweed beetle (adults)	*Tetraopes tetrophthalmus (Forst)*	Coleoptera
15. Milkweed beetle (adults)	*Chrysochus auratus Fab.*	Coleoptera
16. Gypsy moth (adults)	*Porthetria dispar (L)*	Lepidoptera
17. Gypsy moth (pupae)	*Porthetria dispar (L)*	Lepidoptera
18. Forest tent caterpillar (adults)	*Malacosoma disstria Hbn.*	Lepidoptera
19. Forest tent caterpillar (cocoons)	*Malacosoma disstria Hbn.*	Lepidoptera
20. Eastern tent caterpillar (larvae)	*Malacosoma americanum (F)*	Lepidoptera
21. Cabbage butterfly (adults)	*Pieris rapae (L)*	Lepidoptera
22. Cabbage butterfly (larvae)	*Pieris rapae (L)*	Lepidoptera
23. Monarch butterfly (adults)	*Danaus plexippus (L)*	Lepidoptera
24. Gnat (larvae)	*Glyptotendipes lobiferous Say.*	Diptera
25. Blackfly (adults)	*Simulium pictipes Hagen*	Diptera
26. Blackfly (larvae)	*Simulium pictipes Hagen*	Diptera
27. Sawfly (larvae)*		Hymenoptera
28. Allegheny mound ant (adults)	*Formica exsectoides Forel*	Hymenoptera
29. Bald-faced hornet (adults)	*Vespula maculata (L)*	Hymenoptera
30. Honeybee (adults)	*Apis mellifera L*	Hymenoptera
31. Honeybee (larvae)	*Apis mellifera L*	Hymenoptera
32. Yellow jacket (adults)	*Vespa arenaria Fabr.*	Hymenoptera
33. Yellow jacket (larvae)	*Vespa arenaria Fabr.*	Hymenoptera

*Species being determined.

The patients on whom we performed our studies were mainly those being treated in our private practices, but a sizeable group of unselected patients was also studied in a large Veterans Administration general hospital. The vast majority of our patients ranged from 25 to 45 years in age, and the group contained a few more men than women. Our results are summarized in Table II.

Table II compares the results of skin testing done on twenty nonallergic persons with results in twenty patients who had active allergic disease. The

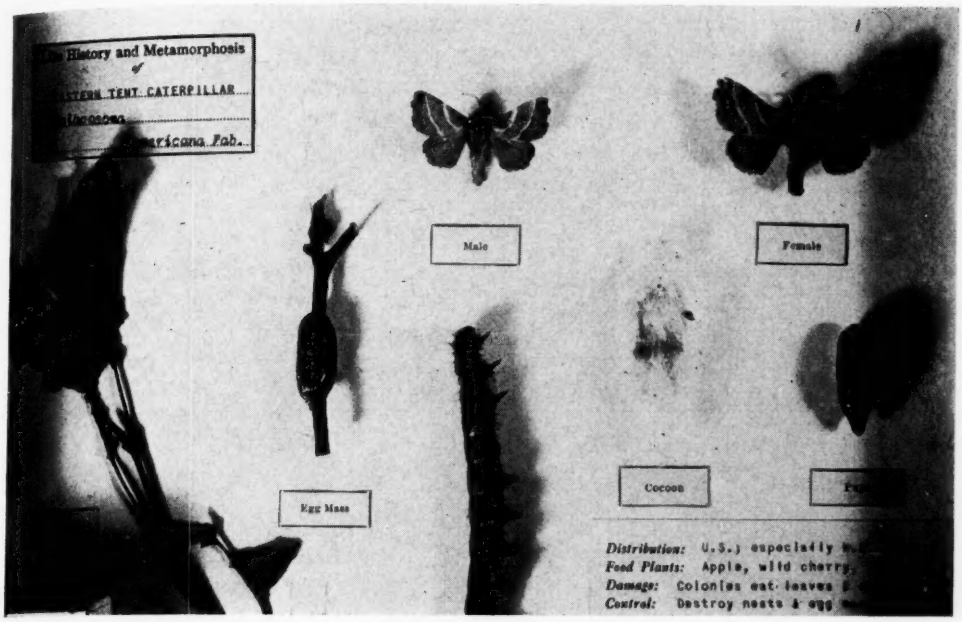

Fig. 1.—Complete metamorphosis.

clinical allergies were diagnosed by skin testing, but the patients had never received any immunization therapy. Only twenty-six extracts were available for testing at the time this portion of the study was undertaken. The results show a striking difference between the number of positive reactions obtained in allergic persons as compared with those obtained in the nonallergic controls. Only sixteen 1 plus reactions were observed in all the nonallergic controls when 100 P.N.U. strength extracts were used, whereas 195 1, 2, and 3 plus reactions were obtained in the allergic patients. When tests with the 5,000 P.N.U extracts were compared, a larger number of reactions was again obtained in the allergic patients. An ever more significant finding, however, was the fact that the allergic patients had so many more 2 and 3 plus reactions than the nonallergic controls. Results of additional studies carried out on another group of forty allergic patients, of whom twenty had seasonal and twenty had nonseasonal symptoms, are summarized in Table III.

Table III shows that pollen-sensitive persons have many more significant reactions to insects than those patients who have nonseasonal symptoms. In

TABLE II. COMPARISON OF TESTS IN ALLERGIC AND NONALLERGIC CONTROLS

TYPE OF PATIENT	100 P.N.U.				5,000 P.N.U.			
	0	+	++	+++	0	+	++	+++
Nonallergic	504	16	0	0	417	98	5	0
Allergic	328	156	32	4	146	193	134	47

+ = Less than 0.5 cm.
++ = 1 cm. wheal or larger.
+++ = 1 cm. wheal with pseudopods.

fact, the pollen group had so many large positive reactions that it was possible to test only a small number safely with 5,000 P.N.U. strength extracts. All of the patients studied whose results are summarized in either Table II or Table III were tested with the same twenty-six extracts, and a like number of tests using comparable strengths was performed on each subject. Representative positive skin-test reactions were confirmed by passive-transfer studies.

TABLE III. COMPARISON OF REACTIONS OBTAINED IN DIFFERENT TYPES OF ALLERGIC PATIENTS

TYPE OF ALLERGIC PATIENT	100 P.N.U.				5,000 P.N.U.			
	0	+	++	+++	0	+	++	+++
Seasonal	182	280	53	5				
Nonseasonal	483	37	0	0	358	150	12	0

\+ = Less than 0.5 cm.
++ = 1 cm. wheal or larger.
+++ = 1 cm. wheal with pseudopods.

Table IV illustrates the reactions obtained in allergic patients after testing with forest tent caterpillar and yellow jacket extracts obtained at various stages of the life cycle of these insects. The scale in Table IV shows how the scores were derived from the positive reactions.

TABLE IV

ALLERGEN	100 P.N.U.		5,000 P.N.U.	
	NUMBER OF PATIENTS	SCORE*	NUMBER OF PATIENTS	SCORE*
Forest tent caterpillar				
Larva	64	34	75	28
Cocoon	45	22	58	25
Adult	46	19	61	32
Yellow jacket				
Adult	37	21	54	35
Larva	36	22	53	33

\+ = 1.
++ = 2.
+++ = 3.
*Score equals total of all positive reactions.

The results show very little difference between reactions in the 100 P.N.U. strength extracts but a slight tendency for increased reactions to the adult form when 5,000 P.N.U. strength extracts were used. We have no explanation of why larger reactions were not always obtained with the 5,000 P.N.U. extracts.

Table V demonstrates comparative reactions obtained to several different members of the order *Coleoptera*.

Essentially the same amount of activity was observed among all the adult beetles, but the cast skins of the carpet beetle larvae appeared to be considerably more antigenic when compared to other adult forms of this order. We emphasize this point because the carpet beetle is potentially one of the

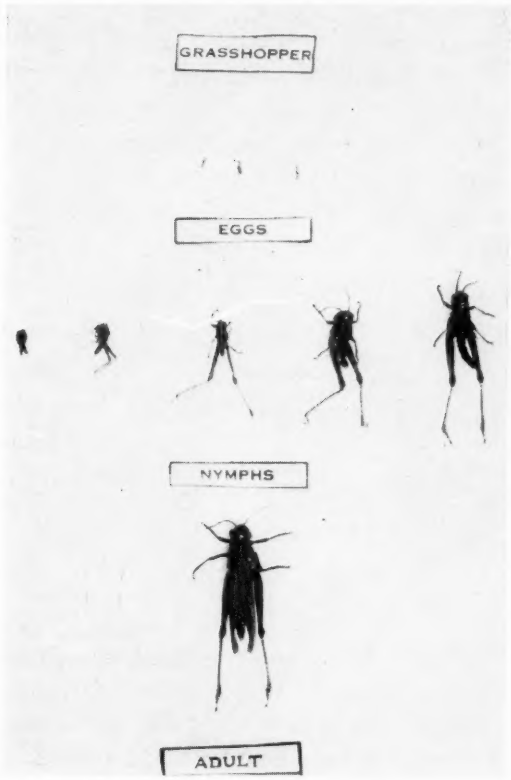

Fig. 2.—Incomplete metamorphosis.

Fig. 3.—Various stages of the life cycle of a carpet beetle compared to a standard millimeter scale. *1*, Larva. *2*, Pupae enclosed in old larval skin. *3*, Empty cast skins. *4*, Adult beetles.

more important insects that we studied. Entomologists state very definitely that these insects are found in 50 per cent of all wool carpets. The larval forms of the carpet beetle shed countless minute skins which may constitute an antigenic stimulant.

Fig. 3 shows the adult carpet beetle, the larva, and the cast skins of the larva. To emphasize how small these specimens are, they have been placed adjacent to a millimeter rule. In view of their size, it is not surprising that patients are usually unaware of the presence of these beetles in their homes.

TABLE V

ALLERGEN	100 P.N.U.		5,000 P.N.U.	
	NUMBER OF PATIENTS	SCORE*	NUMBER OF PATIENTS	SCORE*
Milkweed beetle (adults)	46	21	61	31
Japanese beetle (adults)	42	20	60	30
European chafer (adults)	43	21	60	23
Cast skins of the carpet beetle larvae	34	16	49	38

\+ = 1.
++ = 2.
+++ = 3.
*Score equals total of all positive reactions.

Our studies suggest that common antigens are present in the various stages of the life cycle of the same insect, as well as in different members of the same order, but we feel that our work is still in the preliminary stage and it is too early to draw any definite conclusions.

SUMMARY

1. Insects cause a considerable number of skin-test reactions in most highly sensitive allergic patients.

2. Insect allergy may be a significant causative factor in clinical allergic respiratory disease, especially of the seasonal type.

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THE ISOLATION AND CHARACTERIZATION OF A PURIFIED HOUSE DUST ALLERGEN FRACTION*

WILTON E. VANNIER, M.D., PH.D.,** AND DAN H. CAMPBELL, PH.D.,
PASADENA, CALIF.

THE importance of house dust sensitivity in clinical allergy has been appreciated since the early observations of Cooke.[1] Even though house dust is a very heterogenous mixture clinically, house dust sensitivity appears to be due to a unique allergen or group of allergens.

For many years aqueous extracts of house dust have been used for diagnostic tests and hyposensitization. Boatner and Efron[2] were among the first to attempt to concentrate and study the chemical properties of the skin-reacting materials in house dust. They fractionated an aqueous extract of house dust with dioxane and then with ammonium sulfate, and they obtained a material that was a potent allergen and gave positive tests for both protein and carbohydrate. Sutherland,[3] in 1942, prepared potent extracts of house dust by extraction of the dust with 0.01 N ammonia solution and adsorption of the active material on benzoic acid. The material was found to consist largely of carbohydrate. Rimington and colleagues[4] have carried out further purification by acetone fractionation of dust allergen prepared by Sutherland's method. Their purified allergen fraction contained both carbohydrate and polypeptide. Additional information regarding the composition of this material has been presented as an unpublished communication in a paper by Cayton and associates[5] on a comparison of the properties of extracts of cotton dust and house dust. The purified dust allergen fractions were found to contain 40 to 60 per cent hexose (equivalent galactose by the orcinol method) and 5 to 7 per cent nitrogen. Hydrolysis and paper chromatography indicated the presence of nine aliphatic amino acids, galactose, arabinose, xylose, and a very small amount of rhamnose and glucosamine. The possibility of a trace of galacturonic acid was also mentioned. Rimington[4] also reported the presence of two main electrophoretic components (phosphate buffer, pH 8, $\mu = 0.2$) of about equal skin reactivity and equal nitrogen and hexose contents. On the basis of partial

From the Division of Chemistry and Chemical Engineering, California Institute of Technology.

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**Staff Member, Laboratory of Immunology, National Institute of Allergy and Infectious Diseases assigned to the California Institute of Technology.

hydrolysis studies in which about 80 per cent of the carbohydrate was liberated without loss of amino acids and without loss of skin reactivity, Rimington concluded that the biologic activity was associated with the component containing amino acid rather than the carbohydrate component. On further hydrolysis, skin reactivity was lost when amino acids appeared in the hydrolysate.

We have fractioned aqueous extracts of house dust* and mattress dust† with ammonium sulfate and a variety of organic solvents. In general, most of the fractions were of a similar order of activity by direct skin test. The least soluble materials had the higher nitrogen content (2 to 5 per cent), while the more soluble fractions had lower nitrogen contents (0.3 to 2 per cent). In this article, we describe the isolation and characterization of an active dust allergen fraction,‡ low in both nitrogen and color and consisting predominantly of a heterogenous mixture of acidic polysaccharides.

ISOLATION AND CLINICAL TESTING OF PURIFIED DUST FRACTION

A mixture of mattress dust and house dust was wetted with water, frozen, and thawed, and the fluid was removed with a large mechanical press. The aqueous extract was filtered, concentrated *in vacuo,* and then fractionated at one-half saturation in ammonium sulfate. The material soluble at this salt concentration was dialyzed free of ammonium sulfate and then precipitated three times with methanol. An aqueous solution of the methanol-precipitated material was dialyzed against water, and colored material was removed by

TABLE I. SKIN TESTS REPORTED BY HOLLISTER-STIER LABORATORIES

PATIENT	ENDO 1/1,000	PREPARATION* AND TEST RESULT†			
		CALTECH DUST		H-S DUST	
		1/500	1/1,000	1/500	1/1,000
L.	3	1	1	1	1
C. D.	2	1	1	2	1
F. B.	3	3	2	3	3
S.	2	2	1	2	1
Y.	2	1	2	1	1
M. W.	3	2	1	2	?
F.	4	4	?	3	?
Y.	2	3	0	1	0
D. M.	3	2	2	2	0
K.	1	0	0	1	0
H.	4	3	2	3	1
H. R. S.	3	2	1	1	0
D. H.	3	1	0	0	0
D. L.	4	?	0	4	3
G.	1	1	0	1	?
A.	2	2	1	2	2

*The dust preparations were as follows: Endo, House Dust C ncentrate. Endo Laboratories, Inc.; Caltech Dust, the authors' purified dust extract; H-S Dust, Hollister-Stier Dust Extract. The dilutions were made on a dry weight basis.

†The skin reactions were graded 1+ through 4+. The symbol ? indicates a questionable reaction, and 0 indicates a negative reaction. These were intradermal skin tests.

*Collected and donated by various members of the American Academy of Allergy.

†A pool of about 200 pounds of mattress dust from a mattress-cleaning establishment in Atlanta, Georgia, was sent to us by Dr. McK. Pipies.

‡This is the same dust fraction as the extract C discussed by Follensby, Lowell, and Schiller (J. ALLERGY 27: 103, 1956).

fractional precipitation with basic lead acetate solution.[6] The solution was centrifuged after each addition of basic lead acetate to remove colored material. The lead was removed from the decolorized fraction by means of hydrogen sulfide and centrifugation. The supernatant was dialyzed against water, and the final product was obtained by methanol precipitation. The precipitate was dried and stored in a vacuum desiccator over calcium chloride. A schematic diagram of this fractionation procedure is presented in Fig. 1.

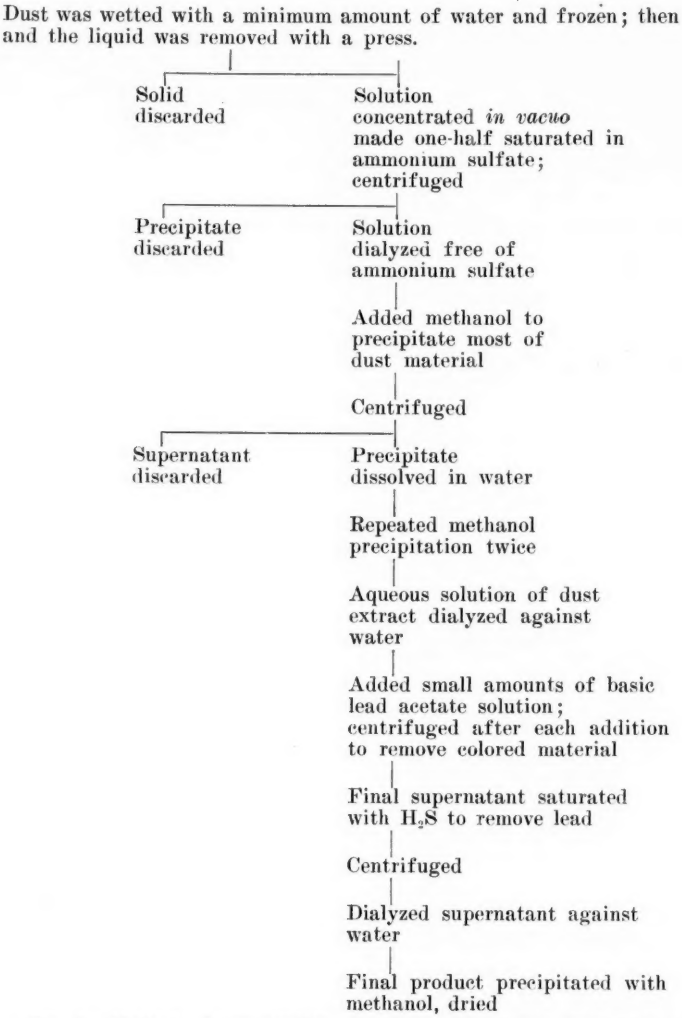

Dust was wetted with a minimum amount of water and frozen; then it was thawed and the liquid was removed with a press.
Solid discarded
Solution concentrated *in vacuo* made one-half saturated in ammonium sulfate; centrifuged
Precipitate discarded
Solution dialyzed free of ammonium sulfate
Added methanol to precipitate most of dust material
Centrifuged
Supernatant discarded
Precipitate dissolved in water
Repeated methanol precipitation twice
Aqueous solution of dust extract dialyzed against water
Added small amounts of basic lead acetate solution; centrifuged after each addition to remove colored material
Final supernatant saturated with H_2S to remove lead
Centrifuged
Dialyzed supernatant against water
Final product precipitated with methanol, dried

Fig. 1.—Method of preparation of the purified house dust allergen fraction.

The Hollister-Stier Laboratories carried out a series of skin tests on patients sensitive to house dust to compare the reactivity of the lead-acetate-purified fraction with two commercial preparations. The data are presented in Table I.

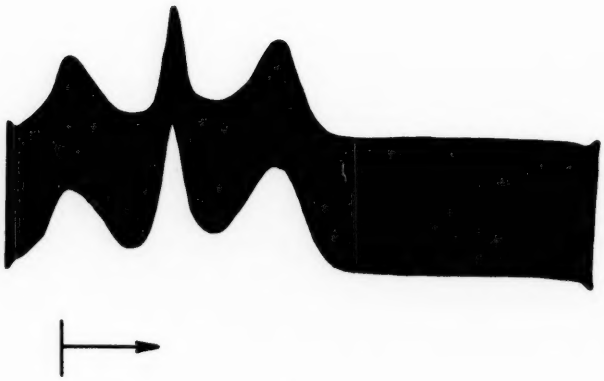
PHYSICAL PROPERTIES OF PURIFIED FRACTION

The allergen fraction was an amorphous white powder which was readily soluble in water to produce a clear, colorless solution.

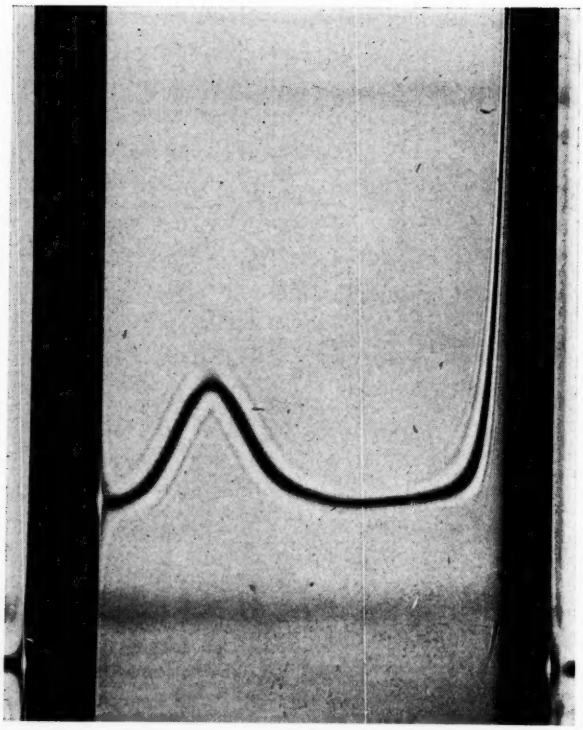
The optical rotation of a 1 per cent solution of the material in water was determined ($[\alpha]_D^{26^\circ} = +63^\circ$). This result was subject to considerable error due to the small amount of material available for the determination.

An electrophoretic analysis of the material was carried out in the Perkin-Elmer electrophoresis apparatus, Model 38. A 2 ml. cell with an open system was used in these experiments. A 1 per cent solution of the dust fraction in acetate buffer (pH 4.7, $\mu = 0.1$) was prepared, and the solution was dialyzed against a 1 liter volume of the buffer. A current of 6.0 Ma. was allowed to pass through the cell for 3,600 seconds, and then pictures were taken by the Longsworth scanning procedure. A reproduction of the pattern obtained from the descending limb is presented in Fig. 2. The fast peak has a mobility of -8.9×10^{-5} (cm.^{2} sec.$^{-1}$ volts^{-1}), while the mobility of the slower peak is -4.6×10^{-5}. Area analysis indicates that about 60 per cent of the material is present in the fast peak (neglecting the ϵ boundary). The stationary or salt boundary (ϵ boundary) probably contains uncharged material as well as the usual salt gradient; however, the magnitude of this effect is not known.

Sedimentation studies were carried out in the Spinco Model E ultracentrifuge; buffer of the same composition as that employed for the electrophoresis experiment was used. A number of runs were made at different allergen concentrations, so that an $S^\circ_{w,20}$ value (sedimentation coefficient extrapolated to infinite dilution) could be obtained. All the samples were run in a synthetic boundary cell, since the sedimentation coefficients were so low that boundaries did not readily form in a regular cell. The buffer against which the sample had been dialyzed was used to form the boundary in the cell. In all cases, the experiments were carried out at full speed (59,780 r.p.m. or approximately 250,000 g) with the temperature of the rotor controlled close to its initial temperature after gaining full speed. Schlieren pictures were taken every eight minutes. From projections of these pictures, the positions of the concentration gradients at various times were determined and the uncorrected sedimentation coefficients were calculated. By the use of Svedberg's tables[7] and the assumed value of 0.6 for the partial specific volume ($\bar{v}$) of the dust material, the sedimentation coefficients were corrected to standard conditions. The value of 0.6 was based on Säverborn's[8] determination of the partial specific volumes of various polyuronides. One of the sedimentation patterns obtained is reproduced in Fig. 2. Each pattern shows a single symmetrical peak. The corrected sedimentation coefficients ($S_{w,20}$) are plotted in Fig. 3, and the extrapolation to infinite dilution to obtain $S^\circ_{w,20}$ (2.6S) is shown. In order to obtain a more nearly linear extrapolation of the $S_{w,20}$ values to $S^\circ_{w,20}$, a plot of the reciprocal ($1/S_{w,20}$) of the $S_{w,20}$ values versus concentration was used to evaluate $S^\circ_{w,20}$ rather than the direct plot shown in Fig. 3.



A.



B.

Fig. 2.—Electrophoretic and sedimentation patterns of a 1 per cent solution of the purified dust fraction in acetate buffer, pH 4.7, μ 0.1. *A*, Descending electrophoretic pattern after 3,600 seconds. *B*, Sedimentation pattern after 2,880 seconds. The vertical line on the electrophoretic pattern indicates the position of the starting boundary, and the arrow shows the direction of motion of the boundaries.

CHEMICAL COMPOSITION OF PURIFIED FRACTION

Elementary Analysis.—A sample of the purified dust fraction was submitted to a commercial laboratory* for analysis. The sample contained 38.37 per cent carbon, 5.98 per cent hydrogen, 1 per cent nitrogen, 7.9 per cent ash, and 54.65 per cent oxygen by difference after ash correction. With the ash correction, and neglecting the nitrogen content, this leads to an empirical formula of the type $[C_{1.09}(H_{2.04}O_{1.00})]_n$. A phosphorus analysis was carried out by the method of Allen.[9] The material was found to contain 0.1 per cent phosphorus. It has been found in the case of other dust fractions that the ash content may be reduced to zero by electrodialysis.

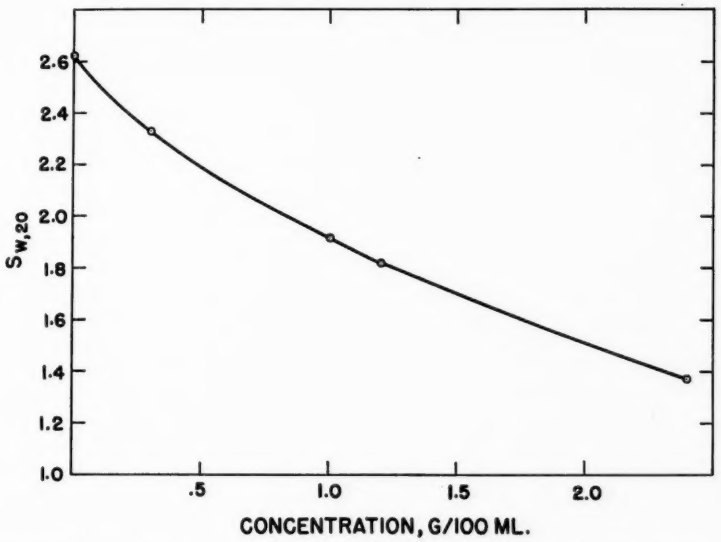


Fig. 3.—Concentration dependence of the sedimentation coefficient ($S_{w,20}$) of the purified dust fraction (acetate buffer, pH 4.7, μ 0.1) with extrapolation to infinite dilution to obtain $S°_{w,20}$.

Hexosamine Estimation.—The Elson-Morgan procedure as modified by Rimington[10, 11] was employed. Weighed samples of the purified fraction (about 12 mg.) were hydrolyzed in sealed test tubes in an oven (105° C.) with 3 ml. of 0.5 N sulfuric acid for eight hours. It had previously been determined by the use of crude dust extracts that these hydrolysis conditions were suitable for the hexosamine determination. The crude dust extracts had been found, by this method, to contain about 2 per cent equivalent hexosamine, while the purified material was found to have 0 per cent equivalent hexosamine.

Determination of Total Reducing Substances.—Weighed samples (about 3 mg.), dissolved in 0.5 N sulfuric acid, were hydrolyzed by heating in sealed tubes in an oven at about 105° C. It was found that a maximum yield of reducing substance was obtained after heating for about 105 minutes. The

*Dr. Adalbert Elek, 4763 W. Adams, Los Angeles 16, California.

samples were analyzed by the method of Hagedorn-Jensen[12] as described by Kabat and Mayer.[13] D-glucose was used as the standard substance for the determination. The maximum yield of reducing substances obtained was 80 per cent (equivalent glucose).

Titration of a Sample of the Purified Dust Fraction.—A carefully weighed sample of the dust fraction (11.6 mg.) was dissolved in 5 ml. of 1 per cent sodium chloride solution. The pH of the solution was measured with a Beckman Model G pH meter and found to be 4.8. A small volume (0.1 ml.) of dilute sodium hydroxide solution was added to make the solution alkaline, and then small volumes of standard hydrochloric acid solution (0.0206 N) were added from a calibrated 2 ml. buret and pH readings were made after each addition. From pH 2.5 to pH 1.7, a more concentrated standard hydrochloric acid solution (0.206 N) was used, so that the volume would not become too large. A blank titration was carried out with 5.0 ml. of 1 per cent sodium chloride solution in the same manner as in the case of the dust fraction. Curves showing the relationship between the pH and the milliequivalents of hydrogen ion added were plotted for both the dust sample and the blank. The difference between the two curves for any particular pH represents the number of milliequivalents of hydrogen ion bound by the dust sample. A plot of the hydrogen ion bound as a function of pH is shown in Fig. 4. The shape of the curve is consistent with the idea that carboxyl groups are being titrated between pH 2.0 and pH 5.5. If we take the number of milliequivalents of hydrogen ion bound between pH 2.0 and pH 5.5 as the acid-binding capacity of the dust sample, then the calculated equivalent weight is 750. If the material were a polyhexose, one out of four of the residues would have a carboxyl group.

Color Reactions for Uronic Acids.—A number of color tests for uronic acids have been explored according to the methods of Dische.[14]

The carbazole reaction[14, 15] for uronic acids was carried out with a 500 γ sample of the purified dust fraction. The corrected spectra obtained with the dust sample and samples of D-glucuronolactone and D-galacturonic acid are presented in Fig. 5. It is not possible to make a quantitative estimate of the uronic acid content of the dust polysaccharides on the basis of such data, as free uronic acid and polysaccharide uronic acid do not necessarily give equivalent color production. The curves obtained with the dust polysaccharide indicate the presence of uronic acid.

A color reaction test for glucuronic acid with thioglycollic acid and sulfuric acid in the presence of mannose[14, 16] was carried out. A 500 γ sample of the purified dust fraction was used for this test with suitable blanks and controls. The resulting spectra showed an absorption maximum at 535 mμ; however, this absorption peak was obscured by a much larger absorption maximum at 610 mμ (Fig. 6, curve *A*). This latter peak is probably due to the presence of other carbohydrate components of the dust fraction. A fraction was obtained from a crude house dust extract that contained the uronic acids with much less of the other sugar components. The dust extract was hydrolyzed by heating in dilute sulfuric acid; then it was neutralized, and the acidic carbohydrate components were removed from the solution by adsorption on a basic

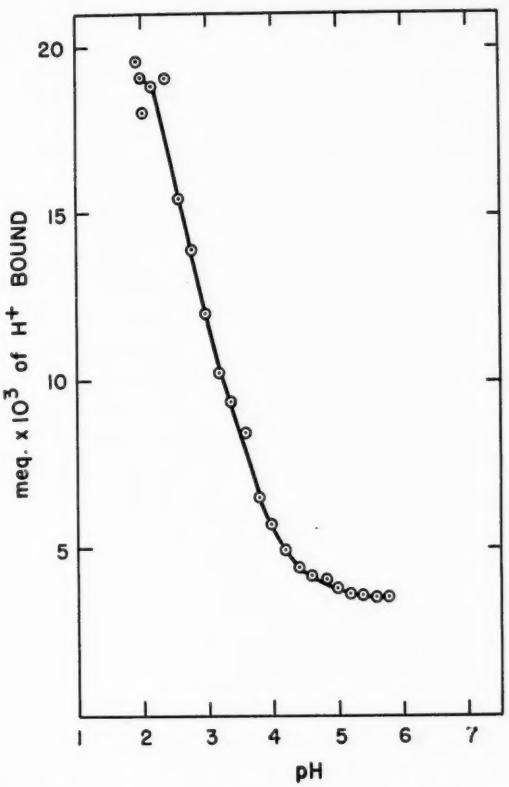


Fig. 4.—Titration curve of a sample (11.6 mg.) of the purified dust fraction.

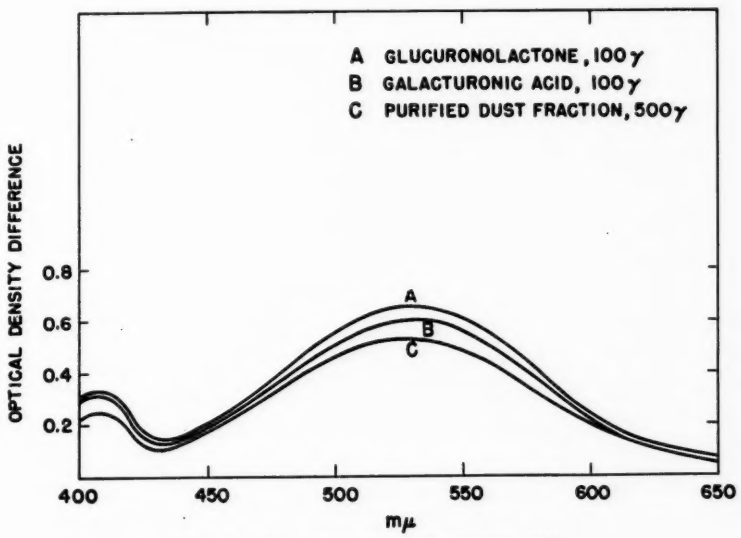


Fig. 5.—Carbazole test for uronic acid.

ion exchange resin column. The column was washed to remove the neutral sugars and the acidic sugar components eluted with dilute acid. This fraction showed an absorption peak at 535 mμ free from the interference of the 610 mμ absorption (Fig. 6, curve *B*).

The cysteine color reaction for galacturonic acid[14, 17] was used to test for the presence of galacturonic acid in the dust fraction. A 2.0 mg. sample of the purified dust fraction was tested by this method and found to be completely negative.

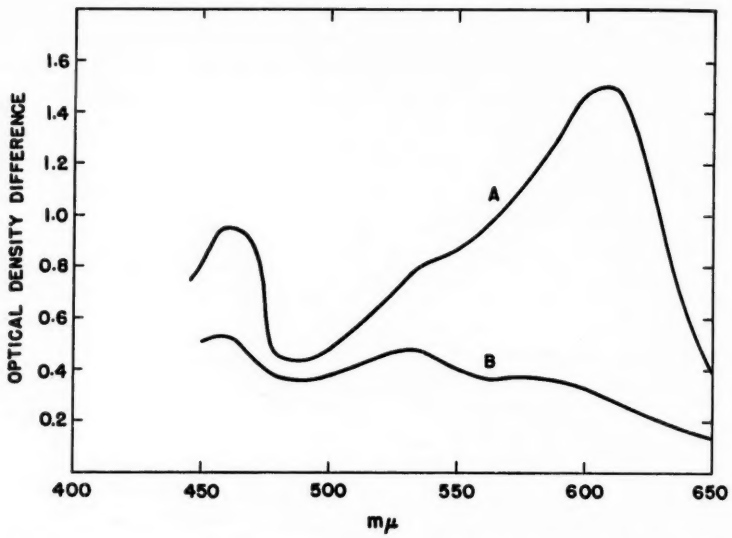


Fig. 6.—Thioglycollic acid test for glucuronic acid. *Curve A*, 500γ of purified dust fraction. *Curve B*, Acidic hydrolysis components from an ion exchange resin column.

Hydrolysis and Paper Chromatography.—Five milligram samples of the purified dust fraction were hydrolyzed by refluxing in 2 ml. volumes of 0.5 N sulfuric acid for two and ten hours. The samples were neutralized with barium carbonate and filtered to remove the barium sulfate and the excess barium carbonate. To concentrate the filtrates to about 0.2 ml., the samples were placed under an infrared heat lamp and a fan. These solutions were used to spot paper chromatograms.

Paper chromatography was carried out by the ascending method described by Williams and Kirby.[18] Sheets of Whatman No. 1 filter paper (36 × 40 cm.) were rolled into cylinders 40 cm. in length and held by four staples. The experiments were carried out in pipette jars (6 × 18 inches) covered with glass plates. The hydrolysates were compared with each other and with known sugars in two solvent systems: pyridine-*n*-butanol-water (2, 3, 1.5 by volume)[19] and water-saturated phenol.[20] A 1.6 per cent solution of *p*-anisidine hydrochloride in ethanol 1.5 parts and *n*-butanol 2 parts, by volume,[21] was used as the spot-locating reagent. The dried sheets were sprayed with the *p*-anisidine solution and then heated in an oven at about 105° C. for five to ten minutes.

It was found that the length of time of hydrolysis (two hours versus ten hours) had no effect on the appearance of the chromatograms. The spots from the dust hydrolysate were compared with the spots from known sugars on the same chromatogram. On this basis, a trial mixture of known sugars was prepared as follows: D-glucose, 10 mg.; D-galactose, 10 mg.; D-mannose, 10 mg.; L-rhamnose, 5 mg.; L-arabinose, 2.5 mg.; and water, 1.0 ml. The relative amounts of the sugars used in the standard solution were based on a visual appraisal of the relative intensities of the spots on the paper chromatogram. A comparison of the sugar spots from the dust hydrolysate with those of the standard solution in the pyridine-*n*-butanol-water and the phenol-water solvents showed agreement in those two solvent systems. As a further check on the identity of the sugar components, these solutions were compared by chromatography in three additional solvent systems: *n*-butanol saturated with water, tert.-pentanol-*n*-propanol-water (4, 1, 1.5 by volume),[22] and ethyl acetate-acetic acid-water (2, 1, 1.2 by volume).[23] The Rf's of the sugars were low in the butanol-water and pentanol-propanol-water solvents. Therefore, the chromatograms were run three times in these solvents. The sheets were dried after each run. As with the first two solvents, the sugar spots from the two solutions showed good agreement as to both position and size of the spot on the chromatogram and color when sprayed with the p-anisidine hydrochloride reagent.

A control experiment was carried out to determine the effect of the hydrolysis conditions and the method of preparation of the samples for chromatography on known sugars. D-glucose, D-galactose, D-mannose, D-glucuronolactone, and D-galacturonic acid were individually carried through the hydrolysis and preparatory procedure. The hexoses were examined chromatographically and were found to be identical to samples not carried through the procedure. There was no evidence of any epimerization. Both the D-glucuronolactone and D-galacturonic acid samples were destroyed during this method of hydrolysis and sample preparation.

PREPARATION OF DERIVATIVES OF SUGARS ISOLATED FROM CRUDE HOUSE DUST FRACTIONS

In order to provide more definite evidence for the identity of the sugars found in the purified dust allergen fraction, derivatives of sugars isolated from house dust extracts were prepared and compared by melting point and mixed melting point with the same derivatives prepared from authentic sugar samples. As sufficient material was not available to carry out these studies with the purified fraction, it was necessary to use crude dust extracts as the source of the sugars for preparation of derivatives. In general, it has been found that substituted hydrazones are easier derivatives to prepare on a 2 to 3 mg. scale than benzimidazoles or osotriazoles. The methods used to prepare the substituted hydrazones were based on the procedure given by Cheronis and Entrikin[24] and Van der Haar.[25] Some of the hydrazines are more or less specific precipitants for a particular sugar under a given set of conditions. In these cases, the

derivative could be prepared from a hydrolysis mixture without preliminary separation of the sugars. In other cases it was necessary to make a separation of the sugars before the preparation of the derivative. The method used to fractionate the sugar hydrolysate involved the use of ascending paper chromatography at elevated temperatures (50 to 60° C.).[21, 26]

Fractionation of a Hydrolysate by Paper Chromatography at 50 to 60° C.— A sample of pooled crude dust extract (ca. 400 mg.) was hydrolyzed by refluxing two hours with 180 ml. of 0.5 N aqueous sulfuric acid. The hydrolysate was neutralized with barium carbonate and concentrated just to dryness. The residue was extracted by mixing with four successive volumes of methanol (100 ml., 50 ml., 50 ml., and 20 ml.). A 20 ml. volume of water was added to the methanol solution, and the volume was reduced to approximately 10 ml. under vacuum. The solution was further concentrated with a stream of nitrogen under a heat lamp to a final volume of 1.2 ml. A 0.1 ml. volume of this concentrated sugar solution was used to make a thin streak 5 cm. from the narrow edge of a 40 × 37 cm. sheet of Whatman No. 1 filter paper. A pad, 3 to 4 cm. wide, of six thicknesses of filter paper was stapled at the top of each chromatogram, and the sheet was folded into a cylinder and stapled. Eight such chromatograms were prepared. They were developed at 50 to 55° C. three times in water-saturated *n*-butanol (saturated at room temperature). The solvent fronts were allowed to ascend to wet the filter paper pads at the top of the chromatograms, and the cylinders were then removed from the jars and dried at room temperature before the next run.

TABLE II. COMPONENTS FOUND IN HYDROLYSATE FRACTIONS OBTAINED BY HIGH TEMPERATURE PAPER CHROMATOGRAPHY

FRACTION	COMPOSITION
I	Rhamnose
II	Fucose trace of rhamnose
III	Arabinose, xylose
IV	Mostly mannose, some araminose
V	Glucose, galactose
VI	Galactose, trace of glucose

Strips 3 cm. wide were cut from both edges of each chromatogram, and the position of the sugar bands was located by use of a spray reagent. With the strip from each edge of the chromatogram serving as a guide, the remainder of the chromatograms was cut in strips so as to provide the maximum separation of the sugars. The corresponding strips from each of the eight chromatograms were extracted with 100 ml. of methanol in a Soxhlet extraction apparatus. Each of the methanol extracts was mixed with 25 to 30 ml. of water and concentrated to a final volume of 5 to 10 ml. by vacuum distillation. The resulting solutions were further concentrated with a heat lamp and a stream of nitrogen to a final volume of 1 to 2 ml. A small sample of each fraction was then chromatographed in the pyridine-*n*-butanol-water solvent following the previously described procedure. The sugars present in each fraction, as determined by the paper chromatograms, are listed in Table II. The same sugars

that were found in the purified dust fraction were found in the crude extract, except that two additional sugars (xylose and fucose) are present in the crude dust hydrolysate.

Optical rotation measurements were made with the aqueous solutions of the fractions I through VI. All the solutions had a positive rotation, and the data were consistent with the presence of the following forms of the sugars in the purified dust fraction: D-glucose, D-galactose, D-mannose, L-arabinose, and L-rhamnose.

Preparation of Derivatives of Mannose: Mannose Phenylhydrazone.—Because of the insolubility of mannose phenylhydrazone, phenylhydrazine has been used for quantitative precipitation of mannose from solutions of sugar mixtures. It was not necessary, therefore, to use the hydrolysate fractions in order to prepare this derivative. A 0.5 Gm. sample of crude dust extract was hydrolyzed by refluxing two hours with 200 ml. of 0.5 N sulfuric acid. The hydrolysate was neutralized with barium carbonate, filtered to remove the barium sulfate and excess barium carbonate, and concentrated to 7 ml. by vacuum distillation; the concentrate was extracted twice with 5 ml. volumes of chloroform to remove any lipid components. Previous experiments had shown that about 2 per cent of the weight of the dust fraction could be extracted from the hydrolysate with chloroform. The sample was concentrated to 1 ml. under a stream of nitrogen and a heat lamp, decolorized by heating with Norite (decolorizing carbon), and centrifuged to remove the Norite. A 0.06 ml. volume of 50 per cent aqueous acetic acid (v/v) and a 0.06 ml. volume of freshly distilled phenylhydrazine were added to the dust hydrolysate solution. The solution immediately became turbid and after one-half hour at room temperature, followed by one-half hour in an ice bath, a brown precipitate had formed. The precipitate was removed by centrifugation, washed three times with cold water, and dried in a vacuum oven at 50° C. The product was found to melt at 184 to 186° C.* A sample of known D-mannose (100 mg.) in 0.4 ml. of water was carried through the same procedure. The product melted at 184 to 185° C. A mixed melting point of the two materials was found to be 184 to 185° C. (literature melting point† D-mannose phenylhydrazone 199° C. recrystallized from 60 per cent ethanol).

Mannose p-Bromophenylhydrazone.—The mannose phenylhydrazone (about 6 mg.) was heated in a boiling water bath with 0.06 ml. of benzaldehyde and 0.12 ml. of water for one hour to decompose the phenylhydrazone and release the mannose. The product was cooled in an ice bath, and the benzaldehyde phenylhydrazone removed by centrifugation. The supernatant was extracted three times with an equal volume of diethyl ether and concentrated under a stream of nitrogen and a heat lamp to a final volume of approximately 0.1 to

*All the melting point determinations have been carried out on a micro hot-stage melting point apparatus.

†All the literature melting points are from Brown, C. A., and Zerban, F. W.: Physical and Chemical Methods of Sugar Analysis (ed. 3, New York 1941, John Wiley & Sons, Inc., pp. 685-688), except those for the 2,5 dichlorophenylhydrazones which are from Mandl and Neuberg.[27]

0.2 ml. An equal volume of absolute ethanol, 6.5 mg. of sodium acetate, and 5.4 mg. of *p*-bromophenylhydrazine hydrochloride were added and the solution was mixed. After two minutes a precipitate formed. The mixture was cooled in an ice bath; the precipitate was collected by centrifugation, washed three times with cold water, and dried in a vacuum oven at 50° C. (melting point 198 to 199° C.). A known sample of D-mannose (10 mg.) was mixed with 15 mg. of sodium acetate, 12.4 mg. of *p*-bromophenylhydrazine hydrochloride, and 0.3 ml. of 50 per cent aqueous ethanol. The product was recovered as in the case of the dust derivative (melting point 199 to 200° C.). The mixed melting point was 199 to 201° C. (literature melting point D-mannose *p*-bromophenylhydrazone recrystallized from 50 per cent ethanol, 208° C.).

The supernatant from which the mannose phenylhydrazone precipitated was centrifuged to remove additional precipitate that had formed and extracted with diethyl ether to remove excess phenylhydrazine. A small sample of this solution was chromatographed. The paper chromatogram showed almost complete depletion of the mannose spot. Over the course of a few days, more precipitate formed and was removed by centrifugation. The supernatant was heated on a boiling water bath with 3 or 4 drops of 50 per cent aqueous acetic acid and 0.03 ml. of benzaldehyde for an hour. An immediate turbidity appeared when the benzaldehyde was added. The mixture was cooled in an ice bath, and the precipitate was removed by centrifugation. The supernatant was extracted four times with two volumes of diethyl ether to remove the excess benzaldehyde. The extracted solution was concentrated and rechromatographed. The paper chromatogram showed the usual dust hydrolysate pattern, except that mannose was completely absent.

Preparation of Galactose Derivatives: Galactose α-Methyl α-Phenylhydrazone.—The sugar solution, depleted in mannose, was concentrated to a volume of 0.3 ml.; 0.1 ml. of 50 per cent aqueous acetic acid and 0.06 ml. of freshly distilled α-methyl α-phenylhydrazine were added, and after ten minutes the precipitate that formed was removed by centrifugation. The precipitate was mixed with a few 0.1 ml. volumes of 30 per cent ethanol and gently heated. Only part of the solid dissolved. The less soluble portion was washed with cold water, dried, and washed with diethyl ether. This material was recrystallized from aqueous ethanol, dissolved in a minimum of absolute ethanol and precipitated by the addition of diethyl ether. The product was washed with diethyl ether and dried (melting point 181 to 183° C.).

A known sample of D-galactose (10 mg.) was mixed with 0.4 ml. of water, 0.1 ml. 50 per cent aqueous acetic acid, and 0.1 ml. of freshly distilled α-methyl α-phenylhydrazine, yielding a white precipitate in one minute. After ten minutes at room temperature, the precipitate was collected by centrifugation, washed three times with water, recrystallized from ethanol (95 per cent), and dried. The product was dissolved in a minimum amount of absolute ethanol and precipitated by the addition of diethyl ether, washed with diethyl ether, and dried (melting point 179 to 181° C.). The mixed melting point with the

dust product was 181 to 183° C. (literature melting point D-galactose α-methyl α-phenylhydrazone recrystallized from 30 per cent ethanol 190 to 191° C.). The galactose α-methyl α-phenylhydrazone derived from the dust extract was decomposed by heating with 0.1 ml. of formalin and 0.3 ml. of water in a boiling water bath for one hour. The solution was taken just to dryness under a stream of nitrogen and a heat lamp. The residue was extracted with two 0.2 ml. volumes of water. The water extract was concentrated to 0.1 ml., and a paper chromatogram was prepared. Only one sugar was found, and this corresponded to galactose in Rf.

Galactose o-Tolylhydrazone.—An attempt was made to prepare this derivative from the galactose obtained by the decomposition of the α-methyl α-phenylhydrazone, but this was unsuccessful. Therefore, one-half of the galactose fraction VI (Table II), obtained by paper chromatography at 50 to 55° C., was used. This material had been shown by paper chromatography to be almost all galactose. The solution was concentrated to approximately 0.2 ml. under a stream of nitrogen and a heat lamp, and 10 mg. of sodium acetate, 12 mg. of *o*-tolylhydrazine hydrochloride, and 0.3 ml. of 50 per cent aqueous ethanol was added. The solution was heated in a boiling water bath for fifteen minutes and then allowed to cool. A small volume (0.2 ml.) of water was added, and the solution became cloudy. After the volume was reduced by about 20 per cent under a stream of nitrogen and a heat lamp and cooling in an ice bath, yellow crystals were obtained. The product was washed twice with cold water, recrystallized from ethanol (95 per cent), washed with cold ethanol (95 per cent), and dried (vacuum oven 50° C.). The product melted at 167 to 169° C.

D-galactose *o*-tolylhydrazone was prepared from a known sample of D-galactose (10 mg.) by the procedure described above. The known derivative melted at 171 to 172° C. The mixed melting point with the dust product was 170 to 171° C. (literature melting point D-galactose *o*-tolylhydrazone recrystallized from 95 per cent ethanol, 176° C.).

Preparation of a Derivative of Arabinose: Arabinose Diphenylhydrazone.—Fraction III (Table II) from the high-temperature paper chromatography fractionation was used as the starting material for the preparation of this derivative. Paper chromatography had indicated that the fraction contained about equal amounts of arabinose and xylose. The amount used contained roughly 5 mg. of arabinose in a volume of 0.1 ml. An equal volume of absolute ethanol, 5 mg. sodium acetate, 9 mg. diphenylhydrazine hydrochloride, and 0.2 ml. 50 per cent aqueous ethanol were added, and the solution was heated for fifteen minutes in a boiling water bath. The mixture was allowed to cool, the volume was reduced by about 20 per cent under a stream of nitrogen and a heat lamp, and the sample was then cooled in an ice bath. The resulting crystalline product was collected by centrifugation, recrystallized from absolute ethanol and then from 95 per cent ethanol, and dried (melting point 197 to 199° C.). L-arabinose diphenylhydrazone was prepared from a known sample of L-arabinose by the procedure described above. The known derivative melted at 198.5 to 199.5° C.

The mixed melting point with the dust product was 198.5 to 200° C. (literature melting point L-arabinose diphenylhydrazone recrystallized from 95 per cent ethanol, 204° C.).

Preparation of a Derivative of Rhamnose: Rhamnose 2,5 Dichlorophenylhydrazone.—A sample of crude dust hydrolysate (about 600 mg.) was fractionated by filter-paper chromatography as previously described, except that the procedure was carried out at room temperature instead of at 50 to 55° C. and a different solvent system was used (*n*-butanol-ethyl acetate-methanol-water: 6, 1, 1,3 by volume). A portion (about 5 mg.) of the material recovered from the chromatograms at the rhamnose level was used to prepare the 2,5 dichlorophenylhydrazone.[27] The material was contained in a 0.1 ml. volume of water to which was added 3 mg. of sodium acetate and 0.1 ml. of 95 per cent ethanol. A 0.2 ml. volume of a 50 per cent aqueous ethanol solution containing 2.5 per cent acetic acid and 4.5 mg. of 2,5 dichlorophenylhydrazine was added to the sugar solution and heated, with mixing, in a boiling water bath for ten minutes. The solution was cooled, a 0.2 ml. volume of water was added, and the solution was concentrated under a stream of nitrogen and a heat lamp. There was formed a gummy precipitate, which was discarded. Further concentration yielded a crystalline product which was washed three times with cold water and dried and then washed three times with cold diethyl ether and dried. The product was recrystallized from 50 per cent aqueous ethanol, yielding a product that melted at 163.5 to 165.5° C. A known sample of L-rhamnose (about 2 mg.) was carried through the same procedure and yielded a product melting at 168 to 169° C. A mixed melting point was 165 to 168° C. (literature melting point L-rhamnose 2,5 dichlorophenylhydrazone, 171° C.).

Preparation of a Derivative of Glucose: Glucose 2,5 Dichlorophenylhydrazone.—A sample of material (about 4 mg.), chromatographically identical with pure glucose, was isolated from the same dust hydrolysate as the rhamnose sample by repeated preparative paper chromatography in the *n*-butanol-ethyl acetate-methanol-water solvent system. The sample was mixed with 4.5 mg. of 2,5 dichlorophenylhydrazine in 0.1 ml. of methanol and heated for ten to fifteen minutes in a boiling water bath. After being dried and washed with diethyl ether, the material was dissolved in 0.05 ml. of methanol and precipitated by the addition of 1 ml. of diethyl ether and cooling at –15° C. The product was collected by centrifugation, washed with diethyl ether, and then recrystallized twice from a 50 per cent ethanol-carbon tetrachloride solution. The resulting material melted at 148 to 150° C. A known sample of glucose was carried through essentially the same procedure and yielded a product melting at 154 to 155° C. A mixed melting point was 150 to 154° C. (literature melting point D-glucose 2,5 dichlorophenylhydrazone, 160° C.). An aqueous solution of the glucose isolated from the dust hydrolysate was compared in optical rotation and found to be identical with an authentic sample of D-glucose.

DISCUSSION

A purified dust allergen fraction has been isolated from crude dust extracts. This material has been found to give impressive skin reactions with

many, but not all, of those persons who react to the crude dust extracts. A detailed comparative analysis of the data is impossible because the concentrations of the preparations tested are unknown and because complete titrations to end points were not carried out. The fact that many of the patients gave strong reactions to all three of the dust fractions tested indicates that our purified fraction does exhibit good allergen skin reactivity in many dust-sensitive persons. It has been found that some of those persons who reacted to the commercial preparations did not react to our purified material. This could be due to the particular relationship, in the various preparations, between the concentrations of materials of the same allergen specificity and the patient's threshold of skin reactivity. Another more likely explanation is that more than one allergen specificity is involved and that there are materials with allergen specificities in the less purified commercial preparations that are not present in our material. It is reasonable that clinical house dust allergy may be associated with a number of different allergen specificities. It is also possible, however, that some of the reactions obtained to the less purified fractions are due to the presence of other known allergens (for example, animal danders or pollens in the original house dust). The type of dust used to prepare the extracts may also be a factor. Our starting material contained large amounts of mattress dust which may be deficient in some of the allergen specificities normally found in house dust.

The purified dust allergen fraction consists predominantly of a heterogeneous mixture of acidic polysaccharides. This heterogeneity is indicated by the presence of two mobile peaks on free boundary electrophoresis. It is probable that there is also present uncharged material that contributes to the ϵ boundary. The patterns obtained by sedimentation analysis consist of single symmetrical peaks. Upon more detailed analysis of the time dependence of the boundary spreading by the method of Baldwin and Williams,[28, 29] however, it is clear that the allergen fraction consists of a population of materials of varying sedimentation coefficient. The concentration dependence of the sedimentation coefficient, as shown in Fig. 3, is fairly small. This indicates that the molecules are probably fairly symmetrical but does not suggest a choice as to the correct model (random coil, short rod, or a compact globular structure).

The polysaccharide nature of the dust allergen fraction is indicated by the high yield of equivalent glucose (80 per cent) on hydrolysis and by the paper chromatography studies. By hydrolysis and paper chromatography, D-glucose, D-galactose, D-mannose, L-rhamnose, and L-arabinose were tentatively identified as the sugars present in greatest amount. The configurations indicated are based on rough optical rotation measurements of the eluates from the high-temperature chromatograms (see Table II). The identification of these sugars has been confirmed by the preparation of substituted hydrazone derivatives and the comparison, by melting point and mixed melting point, of these dust derivatives with the same derivatives prepared from authentic sugar samples. Substituted hydrazones are not ideal derivatives in that they tend to decompose on melting, and the melting point depends on both the solvent

used in recrystallization and the age of the derivative. Due to the small amounts of material involved, the number of times that the products could be recrystallized was limited. In a number of cases further recrystallization failed to yield any product. These difficulties account for the fact that the melting points of the derivatives are considerably lower than that reported in the literature, even in the case of derivatives prepared from known pure sugars. No melting point depressions were observed on mixing the dust derivative with the known derivative. This proved that the tentative identification based on paper chromatography was correct. The possibility of preparing other derivatives, such as benzimidazoles and osotriazoles, was explored. No difficulty was experienced in the preparation of these other derivatives on a 50 to 100 mg. scale; on proceeding to a 2 to 3 mg. scale, however, it was found that crystalline products could not be consistently isolated.

The acidic nature of the polysaccharides has been shown by titration (Fig. 4) and electrophoresis. The evidence that the acid components are uronic acids and, more specifically, largely glucuronic acid rests on reactions in concentrated sulfuric acid with various reagents to yield characteristic absorption spectra. The absorption curves obtained with the carbazole reaction (Fig. 5) indicate the presence of uronic acid and suggest that the uronic acid content is of the order of 20 per cent. This is in agreement with the result obtained by titration (that is, an equivalent weight of 750). The color reaction with thioglycollic acid (Fig. 6) suggests the presence of glucuronic acid, while the cysteine reaction indicates that all the uronic acid present could not be galacturonic acid. These experiments provide presumptive qualitative evidence for the presence of uronic acid residues, probably glucuronic acid, in the dust fraction.

Rimington[4, 5] has studied the amino acid composition of his dust allergen fraction and, on the basis of his studies, it seems likely that most of the nitrogen present is due to a polypeptide component. We have obtained evidence for the presence of a small amount of hexosamine (1 to 2 per cent) in some crude dust fractions; however, the purified dust fraction appeared to be free of hexosamine.

On the basis of these results, the composition of the purified dust allergen fraction (neglecting the ash content) was roughly estimated to be as follows:

Polysaccharide components	95%
D-glucose	20%
D-galactose	20%
D-mannose	20%
L-rhamnose	10%
L-arabinose	5%
Uronic acid (probably glucuronic acid)	20%
Polypeptide components	5%

The relative amounts of the sugars were based on a visual appraisal of the intensities of the spots and were therefore very crude estimates. The general

chemical composition of this purified fraction is similar to that of most crude dust fractions, except that the latter generally have higher nitrogen contents, some hexosamine by the Elson-Morgan color reaction, and fucose and xylose as demonstrated by paper chromatography.

One of the central problems is that of whether the specific dust allergen activity is due to the presence of a polypeptide component, to a polysaccharide alone, or to a complex molecule containing both moieties. The purified fraction described in this article had a nitrogen content of about 1 per cent, while Rimington's active partial hydrolysis product[4] contained 10 per cent nitrogen and 12 per cent carbohydrate by orcinol determination. In neither case has allergen activity been found to be associated with a carbohydrate or polypeptide component alone. Rimington carried out an experiment in which he followed, by paper chromatography and skin test, the partial acid hydrolysis of his purified allergen fraction. With 80 per cent of the total carbohydrate liberated as free reducing sugar, there was no loss of skin reactivity; however, sudden loss of activity occurred when, upon further hydrolysis, amino acids were split off. The data were interpreted as indicating that the polypeptide portion of the allergen fraction was responsible for the skin activity. It is possible that the data could be explained as well on the basis of continued polysaccharide hydrolysis and uronic acid decarboxylation.

Dr. Michael Heidelberger tested the purified fraction for cross-reactivity with various pneumococcus polysaccharides. There was a strong cross-reaction with type-specific substances VII, XII, XV, and XVIII. There were more moderate but definite cross-reactions with twelve of the other type-specific substances. Some of the moderate reactions were negative at low concentration but became positive at higher concentrations. This was interpreted as indicating that the material was heterogeneous. The dust fraction gave a moderate cross-reaction with type-specific substances II and VIII, both of which contained glucuronic acid, glucose, and rhamnose. There was only a very slight cross-reaction with type III (polycellobiuronic acid). The general conclusion drawn was that the dust allergen fraction reacts with many of those antipneumococcus sera that show cross-reaction with glycogen and polyglucoses.

Rimington[4] has reported that his purified allergen specifically inhibited both A and B hemagglutination. Dr. Elvin Kabat[30] tested our purified fraction and found it to be devoid of either of these activities.

The problem of the origin of the specific house dust allergen activity is most intriguing. If the dust allergen activity can be produced under sterile conditions from autoclaved samples of cotton linters,[31, 32] it is difficult to see how a complex polysaccharide or polypeptide could be built up under these conditions. It is much more likely that only a minor change, possibly involving air oxidation, is necessary to convert an inactive material to an active one. A change in a preformed polymer involving some kind of degradative process (possibly oxidative) would tend to produce a polydisperse mixture such as has been found. It is entirely possible that many of the components of such a

polydisperse mixture would possess activity. Cotton fibers consist largely of cellulose, but other constituents are also present.[33] Waxes, protein, pigments, and pectins have been demonstrated. The pectins (partially methyl-esterified polygalacturonic acid with arabinose and xylose components) are present to the extent of about 1 per cent in mature fibers. Possibly some of the arabinose found in the purified fraction and the arabinose and xylose found in less purified materials comes from this source. Some of the uronic acid present could be galacturonic acid; however, it is not likely to be all galacturonic acid, in view of the negative test.

Polysaccharides containing residues of the types found in the house dust fraction have been isolated from a wide variety of bacteria (for example, pneumococci and soil bacteria).[34] Experiments have demonstrated the importance of molds in the production of kapok allergen.[35] It is therefore natural to suspect that bacteria and molds might be involved in the production of the dust allergen. They might either produce the allergen directly or promote the degradation of cellulose or other materials to form the active allergen. More detailed studies with systems in which inactive known materials develop allergen activity, both under sterile conditions and in the presence of bacteria and molds, might aid in solving the problem of the origin and nature of the house dust allergens.

Even though the problem of the chemical nature of the specific house dust allergen has not been solved, these studies have revealed new information about the nature of the materials that have been used clinically for the past thirty years.

SUMMARY

1. An active house dust allergen fraction, low in nitrogen and colorless, has been isolated.

2. The material has been found to show activity with most, but not all, persons sensitive to less purified commercial preparations. This suggests the presence of more than one allergen specificity.

3. The dust fraction was found to be heterogeneous by electrophoretic and sedimentation analysis. Two main peaks were demonstrated by electrophoresis. The average sedimentation coefficient ($S^{\circ}_{w, 20}$) was found to be 2.6S.

4. The chemical composition was found to be roughly 5 per cent polypeptide and 95 per cent polysaccharide, with about equal amounts of uronic acid (probably glucuronic acid), D-glucose, D-galactose, and D-mannose as well as lesser amounts of L-rhamnose and L-arabinose.

5. The relationship of the composition and physical properties of the dust allergen fraction to current ideas concerning the origin of the house dust allergen was discussed.

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VOLUMETRIC COUNTS OF POLLEN GRAINS AT CARDIFF, 1954-1957

H. A. Hyde, M.A. (Cantab.), F.L.S., Cardiff, Wales

INTRODUCTION

The Hirst Automatic Volumetric Spore Trap (AVST).—It is generally agreed that allergic sensitivity to any particular kind of spore is usually acquired by inhaling it from the atmosphere and that both the diagnosis of allergic disease due to such inhalants and the assessment of the effects of its treatment demand a reasonably exact estimate of exposure. For all these reasons, allergists have long sought some means of obtaining a continuous record of the volumetric spore content of the atmosphere.

Scheppegrell[27] attempted, by applying Stokes's formula, to convert "gravity" slide figures directly into volumetric counts. His computations were corrected by Cocke.[2] Pollen grains are not, however, the smooth spheres which Stokes's formula presupposes. Dyakowska[10] found that pollen grains of different shapes but of approximately the same average diameter had widely different rates of fall. Dahl and Ellis,[4] for this and other reasons, deprecated the publication of volumetric computations unless they were accompanied by observed data expressed in terms of grains caught on unit area of exposed surface. Schmidt,[26] however, had pointed out many years earlier that, far from being still or in a state of streamlined motion, as Scheppegrell and others had presupposed, the outdoor air is in a constant state of turbulence; except for a very shallow layer close to the ground, "stokesian" conditions do not obtain outdoors,[12] and calculations based on the supposition that they do are fallacious. Cocke[3] devised and operated a volumetric apparatus and concluded from concurrent observations with gravity slides that his formula[2] for converting gravity slide counts into volumetric concentrations gave values which deviated up to 56 per cent from the actual concentrations obtaining. Durham[5] carried out similar calibration experiments with an impingement apparatus devised by Hawes and associates[15] and later, using the data so obtained as a "pivot," applied his own rate-of-fall figures[6] to compute volumetric estimates based on gravity slide counts.[7] Neither the forms of volumetric apparatus referred to, nor Hill's dust pump, also used by Durham,[8] seems to have been used to give average volumetric incidence over a whole season. Durham[9] later made computations with a view to expressing seasonal incidence in numerical terms, but they were not based on direct volumetric measurement. Gilbert[11] bubbled air at a measured

From the National Museum of Wales and the Asthma and Allergy Research Unit, St. David's Hospital, Cardiff, Wales.

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rate through a collecting solution to obtain estimates of volumetric incidence of ragweed and oak pollen grains at Columbus, Ohio, and compared simultaneous gravity slide counts.

A new approach to the volumetric estimation of airborne particles was made by May,[25] who utilized the principle that the numbers of airborne particles can be estimated ideally by means of a sampling tube, provided that the "orifice with a feathered leading edge faces upstream with suction applied at such a rate that the air velocities in the tube and the main air stream are equal." This principle—that of isokinetic sampling—was embodied by May in his "cascade impactor." In this apparatus a stream of air passes through a succession of sampling tubes and is impacted on a succession of fixed slides on which are caught particles of decreasing orders of size. The same principle was followed by Hirst[16] in his automatic volumetric spore trap, in which a single slide, coated with adhesive and mounted on a carrier actuated by clockwork, moves at the rate of 2 mm. an hour in front of a rectangular orifice measuring 14 by 2 mm. and therefore receives on its surface during twenty-four hours a band-shaped trace 14 mm. wide and 48 mm. long. The air flow is kept constant at 10 liters per minute, which corresponds with a wind speed of 6.0 meters per second or approximately 13.5 miles per hour. This slide is changed daily and, when scanned lengthwise, gives counts which correspond with the average volumetric content for the whole of the twenty-four hours. In theory, the counts made on scanning the slides should be corrected for the actual wind speed obtaining during the operation of the apparatus, and these corrections have been determined for two types of spore by actual calibration using a wind tunnel.[1] Since every type of spore would need its own table of corrections, however, it has been found more convenient to use the uncorrected figures, which in practice are known to be conservative.[18] The inventor of the apparatus has published volumetric counts of pollen grains (undifferentiated) and of various kinds of fungus spores obtained by short runs with his apparatus at Harpenden, England[17] and, in collaboration with Gregory, by a summer run also at Harpenden.[13] Gregory and Sreeramulu[14] have obtained similar series of observations in an estuarine area in southern England. Hamilton[14a] has reported on pollen and spore counts from London and Harpenden, Herefordshire, England.

Operation of Hirst AVST at Cardiff.—A Hirst trap was operated almost continuously from late April, 1954, until the end of the pollen season of 1957 at the pollen observatory on the roof of the National Museum of Wales, Cathays Park, Cardiff. The observatory is 60 feet above ground level, and the museum is situated at an altitude of 45 feet in an area which to the east and south is largely built up and for three miles to the north and west is suburban in character. The immediate neighborhood contains important sources of pollen, including grasses, weeds, and park trees. The country around Cardiff is mainly agricultural, with grass and arable fields and occasional scattered woods.[20]

Two small modifications of the procedure described by Hirst have been introduced. First, the adhesive employed was glycerin jelly (containing basic fuchsin as a stain) and not petrolatum. Second, the chain to which the slide carrier was originally attached was replaced by a steel violin E string, which was found less liable to accidental breakage.

The exposed slides have all been scanned lengthwise. From the counts thus obtained, the average daily volumetric incidence in grains per cubic meter has been computed. In all, eighty-nine types of pollen grains have been recognized,[25] and some 90 per cent of all grains observed have been determined.

RESULTS OF THE CENSUS

Seasonal Incidence of Thirty Types.—The principal results of scanning the daily slides, year by year, are summarized in Tables I to IV. It has been necessary, for the purpose of delimiting the season for each kind of pollen, to adopt an arbitrary end point; this value has been taken as one pollen grain per cubic meter (corresponding to two grains per 0.9 sq. cm. of adhesive surface on the daily slide). Seasonal incidence (P) of a particular pollen type

TABLE I. ATMOSPHERIC POLLEN AT CARDIFF, 1954 (APRIL 26 ONWARD)
SEASONAL INCIDENCE OF TWENTY-ONE TYPES AS DETERMINED BY DAILY VOLUMETRIC COUNTS

POLLEN TYPE	PERIOD	DAYS (n)	AVERAGE VOLUMETRIC COUNTS IN GRAINS PER CUBIC METER (P)	SEASONAL INCIDENCE $P = n \times P$ (UNITS)
Acer (sycamore, maple)	April 29-May 6	8	1.5	12
Aesculus (horse chestnut)	May 14-24	11	1.0	11
Artemisia (mugwort)	August 4-28	25	1.0	25
Castanea (sweet chestnut)	July 4-August 10	38	3.0	114
Chenopodiaceae (beet family)		0		0
Compositae (excl. artemisia)	May 19-July 7	50	0.5	25
Cyperaceae (sedge family)	May 8-21	14	1.0	14
Ericaceae (heather family)	August 31-Sept. 7	8	0.5	4
Fagus (beech)	May 12-15	4	1.5	6
Gramineae (grasses)	May 3-Sept. 24	145	25.0	3,625
Juncaceae (rush family)	July 12-14	3	1.5	4.5
Pinus (pine)	May 15-June 15	32	5.0	160
Plantago (plantain)	May 5-Sept. 6	125	2.0	250
Platanus (plane)	May 8-23	16	1.0	16
Quercus (oak)	April 30-June 21	53	11.0	583
Ranunculus (buttercup)	June 22	1	1.5	1.5
Rumex (sorrels and docks)	May 26-August 4	71	2.0	142
Sambucus (elder)	June 13-July 3	21	1.5	31.5
Tilia (linden)	July 1-28	28	4.0	112
Umbelliferae (parsnip family)		0		0
Urtica (nettle)	May 29-Sept. 18	113	20.0	2,260

Note: The following types are omitted from this table because in 1954 their respective pollen seasons were more or less complete before April 26: Alnus, Betula, Carpinus, Corylus, Fraxinus, Populus, Salix, Taxus, and Ulmus.

has been computed simply as the product $n \times p$ where n equals number of days from first count of one grain per cubic meter to the last such count in the year concerned and p equals the average mean daily concentration during the season. Some kinds of pollen in certain seasons failed to register a mean

concentration of one grain per cubic meter on any single day; their seasonal incidence for the year(s) concerned has been entered as zero, notwithstanding the fact that grains of the type concerned may have been caught in our apparatus, although in numbers here regarded as negligible.

TABLE II. ATMOSPHERIC POLLEN AT CARDIFF, 1955
SEASONAL INCIDENCE OF THIRTY TYPES AS DETERMINED BY DAILY VOLUMETRIC COUNTS

POLLEN TYPE	PERIOD	DAYS (n)	AVERAGE VOLUMETRIC COUNTS IN GRAINS PER CUBIC METER (P)	SEASONAL INCIDENCE $P = n \times P$ (UNITS)
Acer (sycamore, maple)	May 7-14	8	0.5	4
Aesculus (horse chestnut)	May 12-30	19	0.5	9.5
Alnus (alder)	March 16-April 13	29	4.0	116
Artemisia (mugwort)	July 27-August 23	28	2.0	56
Betula (birch)	April 20-May 12	23	4.0	92
Carpinus (hornbeam)		0		0
Castanea (sweet chestnut)	July 10-August 12	34	3.0	102
Chenopodiaceae (beet family)	July 23-August 27	36	2.0	72
Corylus (hazel)	March 16-April 13	29	2.0	58
Compositae (excl. artemisia)	June 5-August 27	84	0.5	42
Cyperaceae (sedge family)	May 23-June 2	11	1.0	11
Ericaceae (heather family)		0		0
Fagus (beech)		0		0
Fraxinus (ash)	April 13-May 11	29	23.0	667
Gramineae (grasses)	May 4-Sept. 8	128	77.0	9,856
Juncaceae (rush family)	June 30-August 7	39	1.0	39
Pinus (pine)	May 25-June 12	19	6.0	114
Plantago (plantain)	May 21-Sept. 7	110	5.0	550
Platanus (plane)	May 25	1	1.0	1
Populus (poplar)	April 19-28	10	1.5	15
Quercus (oak)	May 9-June 6	29	4.0	116
Ranunculus (buttercup)	May 25-July 9	46	1.0	46
Rumex (sorrels and docks)	May 29-July 30	63	7.0	441
Salix (willow)	April 5-May 11	37	1.0	37
Sambucus (elder)	June 17-July 31	45	2.0	90
Taxus (yew)	March 15-April 24	41	2.5	102.5
Tilia (linden)	July 7-28	22	3.0	66
Ulmus (elm)	March 15-May 12	59	13.0	767
Umbelliferae (parsnip family)	May 30-June 11	13	0.5	6.5
Urtica (nettle)	June 4-Sept. 15	104	41.0	4,264

In Table V the thirty types whose incidence is recorded in detail in Tables I to IV are arranged in descending order of average incidence during the four (or three) years concerned. A gravity slide census covering thirteen years at Cardiff and one to five years at twelve other stations in Great Britain gave very similar results,[22] except that certain types of pollen grain which, by reason of their small diameter, are caught less efficiently on horizontal slides than the average are now seen to stand higher in order of numerical importance than was formerly supposed; this applies to chestnut (Castanea) and, above all, to nettle (Urtica). The source plants of the eight types at the top of Table V may be regarded as the chief hay fever plants of Great Britain.

The range of seasonal incidence registered varies widely in all types. Thus, in Urtica (nettle), Plantago (plantain), Taxus (yew), and Populus (poplar) the highest value is about twice the lowest; in grasses it is three

TABLE III. ATMOSPHERIC POLLEN AT CARDIFF, 1956
SEASONAL INCIDENCE OF THIRTY TYPES AS DETERMINED BY DAILY VOLUMETRIC COUNTS

POLLEN TYPE	PERIOD	DAYS (n)	AVERAGE VOLUMETRIC COUNTS IN GRAINS PER CUBIC METER (P)	SEASONAL INCIDENCE $P = n \times P$ (UNITS)
Acer (sycamore, maple)	May 6-23	18	1.0	18
Aesculus (horse chestnut)	May 15-23	9	1.0	9
Alnus (alder)	March 4-April 14	42	8.0	336
Artemisia (mugwort)	August 7-21	15	3.0	45
Betula (birch)	April 11-May 26	46	38.0	1,748
Carpinus (hornbeam)	April 10-May 1	22	1.0	22
Castanea (sweet chestnut)	July 8-August 15	39	9.0	351
Chenopodiaceae (beet family)	July 24-August 13	21	1.0	21
Corylus (hazel)	March 4-April 7	35	7.0	245
Compositae (excl. artemisia)	June 13-July 19	37	0.2	7.5
Cyperaceae (sedge family)	May 10-June 12	34	1.0	34
Ericaceae (heather family)		0		0
Fagus (beech)	May 4-26	23	11.0	253
Fraxinus (ash)	April 11-May 10	30	8.0	240
Gramineae (grasses)	May 4-Sept. 26	146	28.0	4,088
Juncaceae (rush family)	May 1-16	16	0.5	8
Pinus (pine)	May 16-June 23	39	4.0	156
Plantago (plantain)	May 10-Sept. 2	116	5.0	580
Platanus (plane)	May 10-June 4	26	3.0	78
Populus (poplar)	April 7-26	20	1.5	30
Quercus (oak)	May 7-June 22	47	17.0	799
Ranunculus (buttercup)	May 29-June 11	14	0.5	7
Rumex (sorrels and docks)	May 21-July 29	70	5.0	350
Salix (willow)	March 27-May 10	45	2.5	112.5
Sambucus (elder)	June 12-July 30	49	1.0	49
Taxus (yew)	March 4-May 9	67	5.5	368.5
Tilia (linden)	June 28-July 29	32	3.0	96
Ulmus (elm)	March 4-May 15	73	30.0	2,190
Umbelliferae (parsnip family)	June 1	1	1.5	1.5
Urtica (nettle)	May 31-Sept. 27	120	19.0	2,280

times as high and in certain trees it is very much higher. Over a longer period of years, wider differences might well be observed. These variations are illustrated graphically in the frequency diagrams (Figs. 2 to 9) and especially by those for tree pollens (elm, ash, birch, and oak in Figs. 2, 3, 4, and 6, respectively). The potential significance to allergists of such variations has been indicated in an earlier communication[23]; it should always be borne in mind when the effects of treatment are assessed.

Phenology: The time relations of the various types are set out in the calendar (Fig. 1), in which are indicated the earliest and latest dates, respectively, in any of these four (or three) years on which a count of at least 1 grain per cubic meter was registered, and also the period, if any, during which such a minimum count was registered in each and all of these years. For example, elm pollen was present in 1957 as early as February 2 and in 1956 as late as mid-May, while every year it was present from March 15 to 30, as shown by the black line. Eighteen types out of the thirty exhibited such a *constant period;* three (grasses, plantain, and nettle) had very long ones, but twelve had none. The data here recorded are essentially similar to those obtained in earlier studies based on horizontal slides.[19-21]

Day-to-day incidence with special reference to eight principal types: The day-to-day volumetric counts of all types exhibit considerable oscillations during the course of each season, so that the average figures given in Tables I to V do not nearly state the actual mean daily concentrations to which patients may be subjected from time to time. Details of the atmospheric incidence of the eight principal types during the years 1954 (or 1955) to 1957 are therefore set out graphically in the form of day-to-day frequency diagrams in Figs. 2 to 9. The order is that of seasonal onset. The diagrams may be distinguished according to their general pattern as either compact (symmetrical about a midseason maximum) or diffuse (with no regularly recurring maximum phase).[20]

TABLE IV. ATMOSPHERIC POLLEN AT CARDIFF, 1957
SEASONAL INCIDENCE OF THIRTY TYPES AS DETERMINED BY DAILY VOLUMETRIC COUNTS

POLLEN TYPE	PERIOD	DAYS (n)	AVERAGE VOLUMETRIC COUNT IN GRAINS PER CUBIC METER (P)	SEASONAL INCIDENCE $P = n \times P$ (UNITS)
Acer (sycamore, maple)	May 15	1	1.0	1
Aesculus (horse chestnut)	April 19-May 15	27	1.0	27
Alnus (alder)	March 7-28	22	1.0	22
Artemisia (mugwort)	August 1-22	22	4.0	88
Betula (birch)	March 28-May 25	59	9.5	560.5
Carpinus (hornbeam)		0		0
Castanea (sweet chestnut)	June 26-August 3	36	2.5	90
Chenopodiaceae (beet family)	August 1-12	12	1.5	18
Corylus (hazel)	February 26-April 4	38	1.0	38
Compositae (excl. artemisia)	May 19	1	2.0	2
Cyperaceae (sedge family)	June 4-20	17	0.5	8.5
Ericaceae (heather family)		0		0
Fagus (beech)		0		0
Fraxinus (ash)	March 19-April 24	37	14.0	518
Gramineae (grasses)	April 5-Sept. 26	175	50.0	8,750
Juncaceae (rush family)	May 3-4; June 20-July 10	23	1.0	23
Pinus (pine)	May 10-June 15	37	3.0	111
Plantago (plantain)	April 24-Sept. 23	153	3.5	535.5
Platanus (plane)	April 19-May 5	17	0.5	8.5
Populus (poplar)	March 26-April 14	20	1.5	30
Quercus (oak)	April 16-May 19	34	12.0	408
Ranunculus (buttercup)	May 22	1	1.5	1.5
Rumex (sorrels and docks)	May 21-July 10	51	5.0	255
Salix (willow)	March 12-April 5	25	0.5	12.5
Sambucus (elder)	June 4-29	26	2.5	65
Taxus (yew)	Feb. 1-April 5	64	4.0	256
Tilia (linden)	June 19-July 13	25	7.0	175
Ulmus (elm)	Feb. 1-March 30	58	7.5	435
Umbelliferae (parsnip family)		0		0
Urtica (nettle)	June 4-Sept. 26	115	18.0	2,070

Elm (Ulmus, Fig. 2). The diagrams for the two years, 1955 and 1956, are clearly of the compact type, attaining their maxima very near the middle of the season in both years. The 1957 season was much less intense and did not conform so closely to the same pattern. The differences in dates of onset of the pollen season are to be related to weather during the preceding

TABLE V. ATMOSPHERIC POLLEN AT CARDIFF, 1954 TO 1957
THIRTY MAIN TYPES ARRANGED IN DESCENDING ORDER OF NUMERICAL IMPORTANCE

POLLEN TYPE	AVERAGE SEASONAL INCIDENCE (UNITS)	RANGE OF SEASONAL INCIDENCE (PER CENT OF AVERAGE)	HIGHEST MEAN DAILY (24-HOUR) CONCENTRATION	
			VALUE (GRAINS PER CUBIC METER)	DATE
Gramineae (grasses)	6,580	55-150	594	June 24, 1955
Urtica (nettle)	2,718	76-157	205	Aug. 31, 1954
Ulmus (elm)	848	51-258	251	April 3, 1956
Betula (birch)	800	12-218	299	April 27, 1956
Plantago (plantain)	479	52-121	29	June 11, 1956
Quercus (oak)	476	24-168	77	May 22, 1956
Fraxinus (ash)	475	51-141	142	March 29, 1957
Rumex (sorrels and docks)	297	48-150	50	June 11, 1956
Taxus (yew)	242	43-106	67	March 23, 1956
Castanea (sweet chestnut)	161	47-217	35	July 17, 1956
Alnus (alder)	158	14-213	75	March 19, 1956
Pinus (pine)	135	82-118	39	June 1, 1955
Corylus (hazel)	114	33-215	34	March 19, 1956
Tilia (linden)	112	59-156	24	June 20, 1957
Fagus (beech)	64	0-395	23	May 9, 1956
Sambucus (elder)	59	54-153	12	July 9, 1955
Salix (willow)	54	24-210	13	March 27, 1956
Artemisia (mugwort)	53	47-166	11	Aug. 7, 1957
Chenopodiaceae (beet family)	28	0-257	7	Aug. 10 & 12, 1955
Platanus (plane)	26	4-300	13	May 14, 1956
Populus (poplar)	25	60-120	10	April 5, 1957
Compositae (excl. artemisia)	19	10-221	8	May 19, 1954
Juncaceae (rush family)	19	24-205	8	July 8, 1955
Cyperaceae (sedge family)	17	53-200	5	May 16, 1956
Ranunculus (buttercup)	14	11-328	5	June 17, 1955
Aesculus (horse chestnut)	14	64-193	5	May 12, 1957
Acer (sycamore, maple)	9	11-200	5	May 3, 1954
Carpinus (hornbeam)	7	0-300	4	April 23, 1956
Umbelliferae (parsnip family)	2	0-300	2	June 1, 1955
Ericaceae (heather family)	1	0-400	2	Sept. 7, 1954

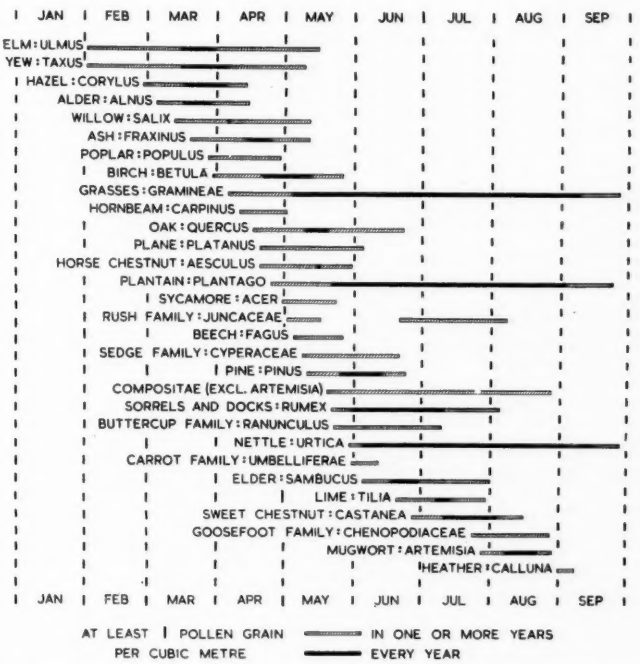

Fig. 1.—Atmospheric pollen calendar for Cardiff, based on direct volumetric counts, 1954-1957. (For further explanation, see text.)

months. The average mean temperature for January and February in 1955 and 1956 was 36° F., but in 1957 it was 42.4° F. The low daily and average concentrations observed in 1957 cannot be explained either by unfavorable weather (there were only three slight frosts after January 19) or by wind direction, which was prevailingly westerly during the ulmus season in all three years; low pollen productivity in that year is doubtless to be related rather to the heavy fruiting observed in 1956 and the consequent exhaustion of reserves.

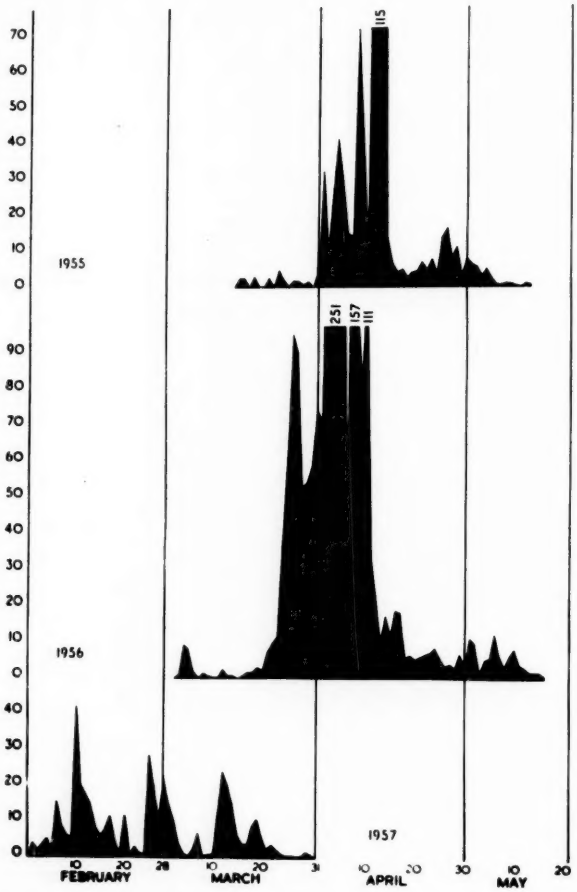

Fig. 2.—Elm (Ulmus) pollen at Cardiff, 1955-1957. Daily mean counts in grains per cubic meter. (For further explanation, see text.)

Ash (Fraxinus, Fig. 3). All the diagrams are compact, with the climax count at mid-season. The wide differences in date of onset and the reasons therefor are similar to those noted above for Ulmus. Here again, relatively low pollen productivity in one year (but not the same year) is to be related to internal causes and not to external influences.

Birch (Betula, Fig. 4). All the diagrams are compact. The contrasted seasons, 1955 and 1956, again illustrate the fact that in some trees a bumper

pollen season may follow or be followed by an almost negligible one. In each year except 1957 the highest concentration was recorded at mid-season.

Grasses (Gramineae, Fig. 5). Daily concentrations exhibit a pattern of rises and falls in which the rises increase up to a maximum before the middle of the season and thereafter decrease (compact type). The peak count

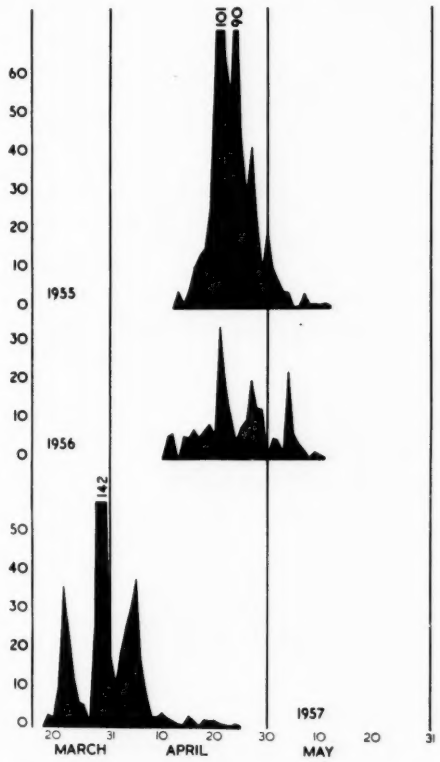

Fig. 3.—Ash (Fraxinus) pollen at Cardiff, 1955-1957. Daily mean counts in grains per cubic meter. (For further explanation, see text.)

always comes between the middle and the end of June—in 1954 on June 22, in 1955 on June 24, in 1956 on June 22, and in 1957 on June 15. Violent day-to-day oscillations in concentration are a normal feature of the grass pollen season in Great Britain; they seem to be related at Cardiff to oscillations in the daily number of hours of bright sunshine.[21a] In 1957, however, there was a period when both these sets of oscillations were suspended. From June 10 to July 6 the weather was unusually sunny; there was an average of 10.2 hours of bright sunshine daily. During this time the pollen count remained well up between 200 and 400 grains per cubic meter and over twenty-seven days averaged 282 per cubic meter. No such period of weather occurred at the same time of year in 1954, 1955, or 1956.

The 1957 grass pollen season also differed from the three preceding it in being virtually complete by July 10. The drought that had gone on

throughout June brought growth (and incidentally flowering) to a stop; rainfall from July 10 caused a resumption of growth but did not result in a recrudescence of grass-pollen fall (contrast with Urtica).

Oak (Quercus, Fig. 6). The diagrams are compact. As in the case of betula, the pollen seasons of 1955 and 1956 form a marked contrast.

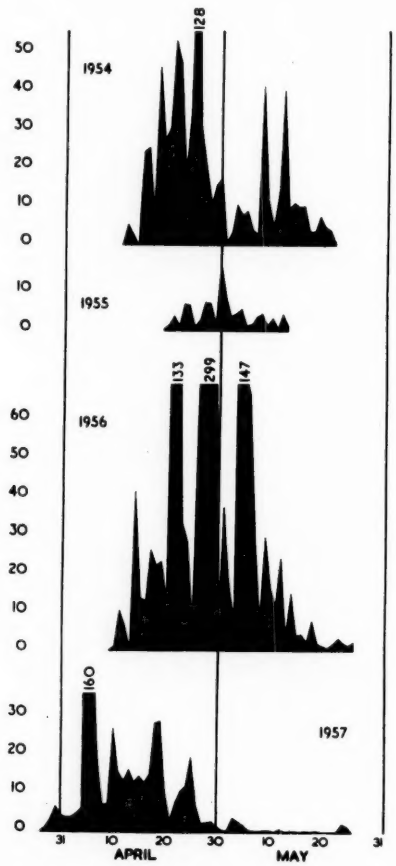

Fig. 4.—Birch (Betula) pollen at Cardiff. 1954-1957. Daily mean counts in grains per cubic meter. Daily concentrations from April 12 to April 25, 1959, have been computed from gravity slide counts (the average relation between gravity slide and Hirst trap counts having been determined for Betula from concurrent exposures at Cardiff, 1955-1957). (For further explanation, see text.)

Plantain (Plantago, Fig. 7). The frequency diagrams are of the diffuse type, although various submaxima occur. Pollen concentration was consistently lower throughout 1954 than in the subsequent years. The opening of the season varied widely, from May 20 in 1955 to April 24 in 1957.

Rumex (sorrels, R. acetosa, and R. acetosella; and docks, especially R. crispus and R. obtusiflorus; Fig. 8). Pollen of *Rumex acetosa* is prevalent during June, that of the docks later, but no recurrent marked maxima can

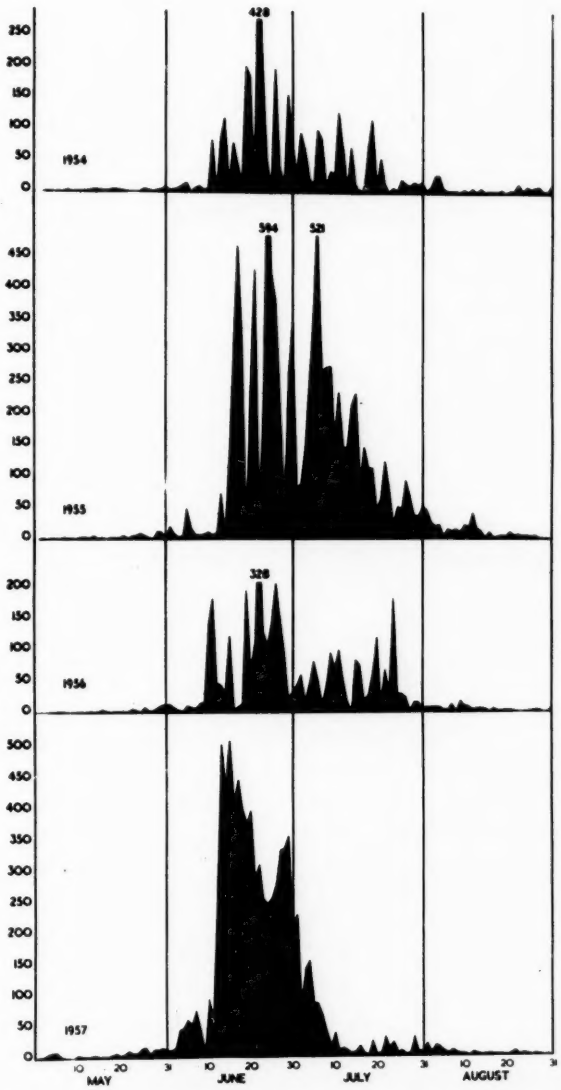

Fig. 5.—Grass pollen at Cardiff, 1954-1957. Daily mean counts in grains per cubic meter. The effective onset of the season and the climax count both come in June every year; in other respects, the 1957 season differed from the three preceding seasons. (For further explanation, see text.)

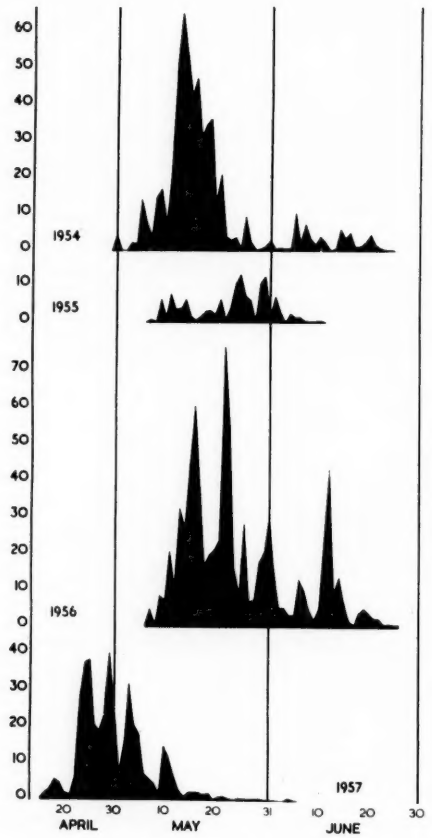

Fig. 6.—Oak (Quercus) pollen at Cardiff, 1954-1957. Daily mean counts in grains per cubic meter. (For further explanation, see text.)

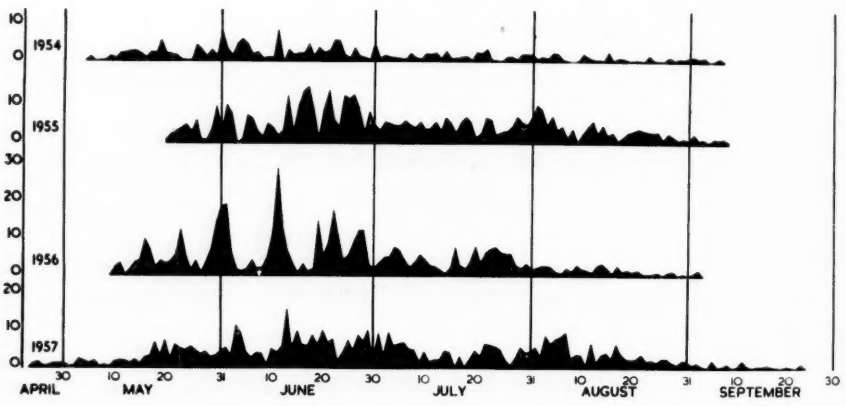

Fig. 7.—Plantain (Plantago) pollen at Cardiff, 1954-1957. Daily mean counts in grains per cubic meter. (For further explanation, see text.)

be related to the seasons of the respective species. The diagrams are of the diffuse type, like those of plantago; here, too, average daily concentrations in 1954 are consistently low.

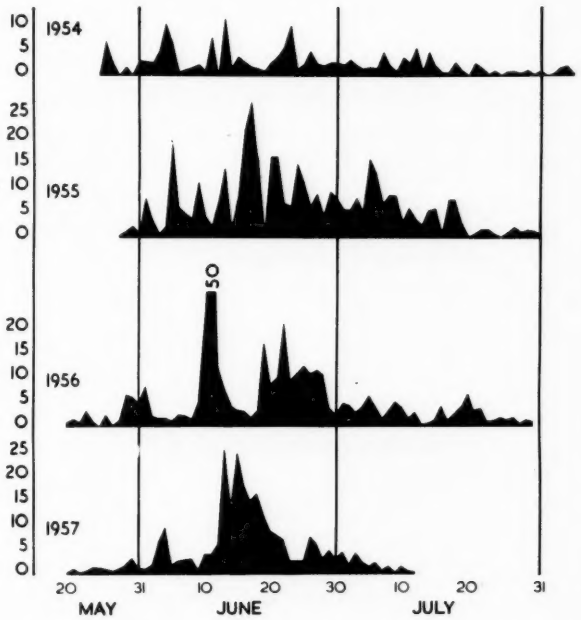

Fig. 8.—Sorrel and dock (Rumex) pollen at Cardiff, 1954-1957. Daily mean counts in grains per cubic meter. (For further explanation, see text.)

Nettle (Urtica, Fig. 9). As already indicated by its position in Table V, nettle pollen is the only kind which during the period concerned gave volumetric counts comparable with those for grass pollen. The frequency diagrams differ from those for grasses in that there is no single peak period which recurs within a particular range of dates; the highest counts occurred in different years on various dates between June 16 and August 31. A preliminary consideration of these diagrams has shown that their general shape is related not to the calendar but to the weather, and in particular to preseasonal and coseasonal rainfall. Thus, the exceptionally dry period, from June 1 to July 6, 1957, followed a relatively dry spring; Urtica pollen release rose steeply after its onset to a moderate level on June 11 and thereafter fell steadily to a very low value on July 27. Rainfall in July permitted fresh growth and a new and higher wave of pollen production, the highest count of the season being attained on August 12 (contrast the grass pollen season for the same year).

The highest mean daily (twenty-four-hour) concentrations of all the thirty types whose incidence is here regarded as significant are stated in the fourth column of Table V.

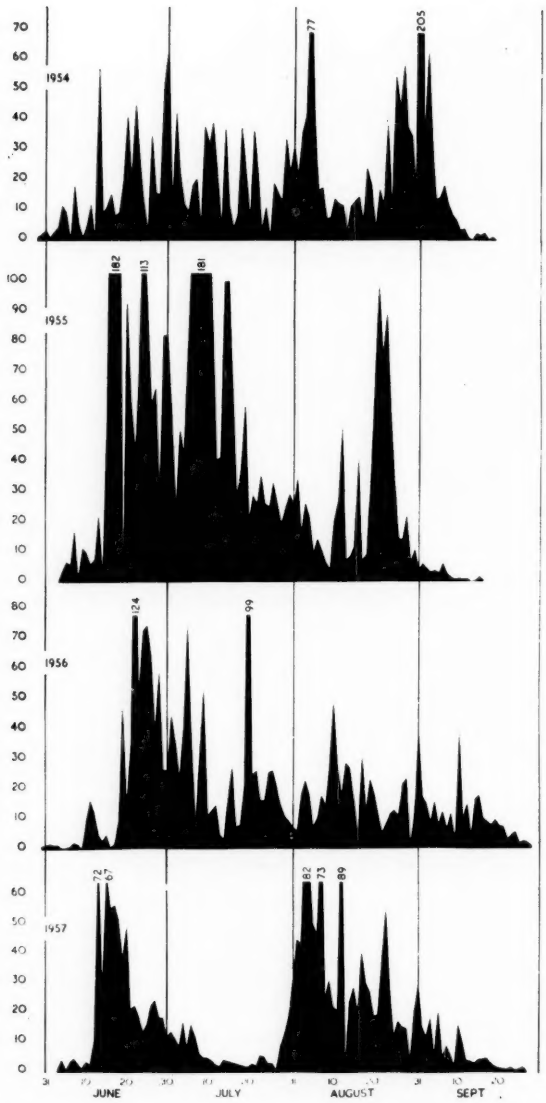

Fig. 9.—Nettle (Urtica) pollen at Cardiff, 1954-1957. Daily mean counts in grains per cubic meter. (For further explanation, see text.)

SUMMARY

1. The article presents the principal results of a four-year pollen census carried out with a Hirst automatic volumetric spore trap.

2. Direct volumetric estimates have been made of the pollen incidence of the various types of pollen concerned; the potential value of such estimates in the study of inhalant allergy needs no emphasis.

3. The survey has confirmed the general conclusions (based on a much longer gravity slide census) on the qualitative and (with the exceptions stated below) the quantitative botanical composition of the pollen cloud over South Wales, on the phenology and (with the same exceptions) the relative abundance of its constituent types, and on their variation from year to year.

4. Use of an efficient volumetric trap has made possible a more correct assessment of the numerical abundance of certain types (notably Urtica) whose relative importance, by reason of their small size, had previously been underestimated.

5. Attention is drawn to especially wide variations in the annual incidence of certain principal types and to the need to consider such variations when the effects of treatment are assessed.

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PARABROMDYLAMINE MALEATE, CHLORPROPHENPYRIDAMINE MALEATE, AND TRIPELENNAMINE HYDROCHLORIDE IN CHRONIC ALLERGIC RHINITIS

WALTER R. MACLAREN, M.D., PASADENA, CALIF.

SINCE the introduction by Halpern,[6] in 1942, of the antihistamines for the treatment of allergic conditions, hundreds of these compounds have been prepared in an attempt to increase specific activity and decrease side effects.

In 1949 and 1950 two propylamines of the class 1-(phenyl)-1-(2 pyridyl)-3-dimethylaminopropane were reported to be "highly effective" therapeutic agents in doses of 2 to 4 mg. against hay fever and other allergic manifestations.[5, 7, 9] Recently a third member of this family, 1-(p-bromophenyl)-1-(2-pyridyl)-3-dimethylaminopropane maleate, or parabromdylamine maleate (Dimetane) has been made available for the symptomatic relief of allergic symptoms.

In animal studies[4] parabromdylamine (maleate) appeared to be equal or slightly superior to its chloro analog, chlorprophenpyridamine, and considerably stronger than tripelennamine or diphenhydramine with respect to protection against histamine-induced bronchospasm; it was also lower in relative toxicity. A clinical evaluation of parabromdylamine has been presented by Thomas,[10] who found that 122 out of 140 patients suffering from various allergic disorders responded satisfactorily to doses ranging from 8 to 32 mg. a day.

In the study reported here parabromdylamine and three new compounds showing antihistaminic activity have been compared with chlorprophenpyridamine and tripelennamine in a group of patients suffering from chronic allergic rhinitis. The new compounds, designated as AHR 209, AHR 211, and AHR 224B, have been prepared by the A. H. Robins Co., Inc. Compared to chlorprophenpyridamine in animal experiments, they are between one-third and one-half as effective against histamine and they are 50 to 100 times as effective as antagonists of acetylcholine.[1-3]

The condition that we have classed as chronic or perennial allergic rhinitis is characterized by edema of the nasal mucus membrane and engorgement of the turbinates, various degrees of nasal obstruction, mouth breathing, some rhinorrhea and postnasal drip, morning sneezing, and usually an increase in pharyngeal lymphoid tissue. Skin-sensitizing antibodies to inhalant allergens are usually present. This condition does not respond readily to antihistamines

From the Department of Medicine, Southern California School of Medicine, and the Allergy Clinics, Los Angeles County General Hospital, Los Angeles.

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and presents a more severe test of medication than the more acute seasonal variety of nasal allergy. In southern California a great many patients with nasal allergy belong in the chronic category, a state explainable by the long growing season and the presence of airborne irritants most of the year. Studies of antihistamines made on these patients tend to yield less dramatic evidence of relief than is reported from other parts of the country.

MATERIALS AND METHODS

Ninety patients suffering primarily or only from chronic allergic rhinitis were selected from the allergy clinics of the Los Angeles County General Hospital and from private practice. In the group were forty males and fifty females, ranging in age from 4½ to 77 years. Nineteen were younger than 12 years. The median age of the adults was 44 years; of the entire group, it was 36 years.

All patients had shown positive skin tests to various inhalant allergens and had been receiving hyposensitization without complete control of symptoms. The average duration of symptoms was 7.5 years. Fourteen of the patients had a history of asthma as well as rhinitis, but none was having active asthma immediately before or during the study. Patients with nasal or sinus infection were excluded.

Parabromdylamine, AHR 209, AHR 211, AHR 224B, and chlorprophenpyridamine were obtained as 4 mg. tablets. Tripelennamine was supplied as a 50 mg. tablet. To conceal the identity of the tablets from both the patients and the observing physicians, each kind of tablet was given a code letter which was used throughout the study.

Patients used each antihistamine for one week on a random system of rotation designed to minimize external factors. Children under 12 were given one tablet of each compound twice a day. In general, adults use one tablet three times a day. A few patients known to be unusually resistant to antihistamines used four to six tablets daily.

All patients cooperated in keeping a "Nasal Allergy Symptom Record"—a chart providing spaces for recording daily the number of sneezing spells, attacks of coughing, itching eyes, the extent of nasal obstruction, and the use of such medication as nose drops. After a minimum of instruction, patients are able to keep a record of symptoms that is much preferable to reliance on memory. From these data, a symptom index was derived by adding the number of symptoms. The patient was asked to give an estimate of improvement in his condition, rated from 0 (no relief) to 4 (complete relief).

The patient's nose was examined at each weekly visit, and, using a pictorial scale as a guide, an estimate was made of the per cent of normal airway present on each side. The average for the two sides was used in tabulation.

Information as to side effects was elicited each week by questioning, care being taken to avoid direct suggestion as much as possible. Sedation is the major complaint with most antihistamines, and the scale, that we have used

before[8] to rate this symptom is as follows: "slight" = scarcely detectable; "mild" = some effort to keep alert; "moderate" = real effort to keep alert, but without complete success; "severe" = practically overcome by sleep. Nervousness and headache were classed as slight or moderate, using the patient's own estimate, as was the case with the other complaints.

RESULTS

The six compounds tested are arranged in Table I in terms of the average weekly symptom index, degree of relief, and per cent of normal nasal airway for the entire group. The data on nasal airway for tripelennamine were incomplete and are not included in the table.

TABLE I. AVERAGE WEEKLY VALUES OF SYMPTOM INDEX, DEGREE OF RELIEF AND PER CENT OF NORMAL NASAL AIRWAY IN NINETY CASES OF CHRONIC ALLERGIC RHINITIS UNDER ANTIHISTAMINE TREATMENT

ANTIHISTAMINE COMPOUND	SYMPTOM INDEX	DEGREE OF RELIEF†	PER CENT OF NORMAL AIRWAY
Parabromdylamine maleate	26.6	2.18	49.6
Chlorprophenpyridamine maleate	25.8	2.22	49.4
Tripelennamine hydrocloride*	22.3	2.28	—
AHR 209	37.2	1.40	40.8
AHR 211	44.4	1.10	38.5
AHR 224B	40.7	1.33	44.1

*Eighty-five cases.
†Complete relief = 4.0; no relief = 0.

For practical purposes, there appears to be no significant difference in effectiveness between 4 mg. doses of parabromdylamine and chlorprophenpyridamine and 50 mg. doses of tripelennamine. The data on parabromdylamine and chlorprophenpyridamine probably reflect the closeness of their chemical structures. The compounds AHR 209, AHR 211, and AHR 224B were not as effective, providing only about half as much relief.

The per cent of normal airway does not seem to be a very sensitive index, although it tends to distinguish between the various antihistamines in the same way as the other indices. Because of the naturally occurring daily variation in nasal obstruction often found, this measurement may, in certain persons, show no correlation with treatment.

Side effects, in general, were mild; only one patient stopped treatment on this account. Table II summarizes the side effects for each compound. Sedation accounted for the majority of the complaints with the three most active antihistamines. For a comparable degree of relief, the use of parabromdylamine resulted in a slightly lower incidence of side effects (particularly sedation) than chlorprophenpyridamine and less than one-half the incidence of side effects produced by tripelennamine. For comparison with another class of antihistamines, it may be mentioned that bromodiphenhydramine (Ambodryl) used in a closely similar group of patients produced side effects in 44.4 per cent of the cases, with no higher degree of relief of symptoms.[8]

TABLE II. NUMBER AND PER CENT OF SIDE EFFECTS FROM PARABROMDYLAMINE, CHLORPROPHENPYRIDAMINE, TRIPELENNAMINE, AHR 209, AHR 211, AND AHR 224B IN CHRONIC ALLERGIC RHINITIS

	PARABROMDYLAMINE (90 CASES)		CHLORPROPHENPYRIDAMINE (90 CASES)		TRIPELENNAMINE (85 CASES)		AHR 209 (90 CASES)		AHR 211 (90 CASES)		AHR 224B (90 CASES)	
SIDE EFFECTS	NO.	PER CENT	NO.	PER CENT	NO.	PER CENT	NO.	PER CENT	NO.	PER CENT	NO.	PER CENT
Sedation:												
Slight	13	13.7	11	11.6	17	18.9	3	3.1	3	3.1	4	4.2
Mild	3	3.1	6	6.3	8	8.9	2	2.1	3	3.1	2	2.1
Moderate	—	—	1	1.1	6	6.7	—	—	—	—	—	—
Total sedation	(16)	(16.8)	(18)	(19.0)	(31)	(34.5)	(5)	(5.2)	(6)	(6.2)	(6)	(6.3)
Nervousness:												
Slight	—	—	1	1.1	1	1.1	1	1.1	2	2.1	—	—
Moderate	1	1.1	—	—	—	—	—	—	5	5.3	1	1.1
Headache:												
Slight	2	2.1	—	—	2	2.2	—	—	2	2.1	3	3.3
Moderate	1	1.1	—	—	—	—	3	3.1	1	1.1	3	3.3
Dizziness	—	—	2	2.1	5	5.5	1	1.1	—	—	1	1.1
Nausea	—	—	1	1.1	4	4.4	—	—	—	—	—	—
Cramps	—	—	—	—	—	—	1	1.1	—	—	—	—
Dryness of nose	—	—	—	—	2	2.2	—	—	—	—	—	—
Totals	20	21.1	22	23.3	45	49.9	11	11.6	16	16.8	14	15.1

The compounds AHR 209, AHR 211, and AHR 224B, in the dosage used, gave low incidences of side effects but correspondingly low degrees of relief.

DISCUSSION

The clinical evaluation of antihistamines depends so much on subjective evidence that it is difficult to be sure of small differences between compounds, even in rather large groups of patients. This is particularly noticeable in dealing with chronic nasal allergy, where complete relief is the exception rather than the rule.

The two ends of the scale of effectiveness—complete relief or none whatever—are fairly definite, but the intermediate grades are subject to coloring by the patient's feelings at the time of questioning, as well as by the interviewer's state of optimism. A numerical count of symptoms, a note of the need for extra medication, and an examination of the offending tissues are of material help in deciding whether the patient's subjective estimate of relief is justified. The more measures that agree, the more reliable are the conclusions drawn.

The results of this study have been presented not as per cent of the group that experienced "excellent," "good," "moderate," or "poor" results but as a single figure representing averages for the entire group. Actual relief was of less interest than comparison of effectiveness and measured side effects for a given degree of relief. As no placebo was used as a control in this study, it was felt that it would be difficult to measure the absolute degree of relief on the medication as compared to none.

In this study parabromdylamine, chlorprophenpyridamine, and tripelennamine showed the same order of effectiveness in controlling symptoms, with

only small difference between the best and the worst in the various indices. For equal degrees of relief, however, the incidence of side effects, especially sedation, with parabromdylamine was less than one-half that found with tripelennamine. This difference was great enough to give parabromdylamine a definite advantage.

The compounds AHR 209, AHR 211, and AHR 224B were much less effective than the three antihistamines just discussed but, because of their low incidence of side effects, could probably be used in higher dosage to advantage. In animal protection experiments, these new compounds were only one-half as effective against histamine as chlorprophenpyridamine, and this is borne out by the clinical data. As acetylcholine antagonists, they were 50 to 100 times as active as chlorprophenpyridamine, which suggests that acetylcholine has little to do with chronic allergic rhinitis.

Even though the two prophylamine antihistamines were closely alike and both were clearly superior to tripelennamine with regard to side effects on the group as a whole, individual preferenee was unpredictable, so that in actual use it will still be necessary to adjust the antihistamine to the patient. Because of the nature of chronic allergic rhinitis, medication has to be used over long periods of time, with concomitant possibility of tolerance and the need for change. Therefore, an effective new antihistamine is a welcome addition to our armamentarium.

SUMMARY

1. Parabromdylamine (maleate), 4 mg., chlorprophenpyridamine (maleate), 4 mg., and tripelennamine (hydrocloride), 50 mg., were found to give closely comparable relief of symptoms in chronic allergic rhinitis.

2. The incidence of side effects with parabromdylamine was slightly less than with chlorprophenpyridamine and one-half as great as with tripelennamine.

3. Three antihistaminic compounds, AHR 209, AHR 211, and AHR 224B in 4 mg. doses were much less effective in relieving symptoms of chronic allergic rhinitis but showed correspondingly low incidence of side effects.

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STUDIES IN TOBACCO HYPERSENSITIVITY

III. Reactions to Skin Tests and Peripheral Vascular Responses

Vincent J. Fontana, M.D., Walter Redisch, M.D., Rose Lee Nemir, M.D., Marjorie K. Smith, M.D., Kurt DeCrinis, M.D., and Marion B. Sulzberger, M.D., New York, N. Y.

THE problem of tobacco hypersensitivity has been the subject of several controversial reports. The investigations of Harkavy, Hebald, and Silbert[1] and of Sulzberger[2] were the first to indicate the possible allergic activity of tobacco as a cause of certain vascular diseases, especially thromboangiitis obliterans. On the other hand, reports by Trasoff and associates,[3] Chobot,[4] and Westcott and Wright[5] failed to substantiate the conclusion that the skin reaction produced by tobacco extract is a specific response designating a sensitization process of the blood vessels.

The contradictory evidence concerning the role of allergy to tobacco as a cause of vascular disease is succinctly summarized by Lowell in a recent work edited by Wynder.[6] An excellent and complete review on the subject of the immunologic aspects of tobacco and smoking has been published by Silvette, Larson, and Haag.[7] The latter authors include in their summary the statement: "A well designed and properly controlled program of clinical investigation of tobacco sensitivity in relation to disease is greatly to be desired."

The divergent views and findings in this field prompted us to investigate further the possibility that certain tobacco effects are based on a specific allergic susceptibility of particular persons rather than on obligatorily toxic products in tobacco smoke. While the observations of Sulzberger, Harkavy, and others have verified the immunologic specificity of the skin reaction to tobacco, the clinical significance of these reactions is not always obvious. That serious difficulties are encountered in the interpretation of skin tests must be admitted and understood. The factors upon which success depends are the reliability of the extracts used, the method of testing, the evaluation of the reactions in a single person, and their correlation with the patient's clinical history. These precautions have been considered throughout this investigation. The extracts used in this study have been prepared by laboratory workers experienced in the field. Care was taken in the use of these extracts and in the performance and

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From the Department of Dermatology and Syphilology, the Department of Pediatrics (Allergy Section), New York University Post-Graduate Medical School, and the New York University Research Service, Goldwater Memorial Hospital, New York University-Bellevue Medical Center.

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reading of the skin tests in order to eliminate or reduce to a minimum the possibility of nonspecific false positive reactions and other results of experimental errors.

MATERIALS AND METHODS

This report includes the skin reactions and historical data on 641 healthy adult volunteers and 294 children tested with tobacco extracts at the Judson Health Center in New York City. The volunteers were skin tested intracutaneously with the five different tobacco extracts. Approximately 0.02 c.c. of the excitant was injected intradermally, and the site was observed for at least twenty minutes to note the result of the test. The skin-test results were interpreted by the standards proposed by Cooke.*

In order to eliminate questionable or irritant reactions, only the moderately and markedly positive reactions were considered positive for the purposes of this study. Permanent tracings were obtained on all marked positive skin tests. Normal saline was used as a control.

The tobacco extracts were prepared by Mrs. Margaret B. Strauss, Director, Allergy Laboratory, University Hospital, New York City. Burley, Virginia, and Turkish cured tobaccos were procured from a reputable tobacconist who could assure their purity of type for the preparation of these individual tobaccos. Mixed tobacco extracts were composed of the following popular brands of cigarettes in equal parts (including the tobacco and the cigarette paper): Camel, Chesterfield, Lucky Strike, Old Gold, Pall Mall, and Philip Morris. No filter cigarettes were used.

The usual routine in preparing an extract of allergen is to treat the crude mass with an organic solvent, such as acetone, ether, toluene, or Sovasol in order to remove as much oil, fat, and coloring matter as possible before the aqueous extraction. In general, the oil and dye contain no antigenic fraction important to the inhalant type of allergies and their removal produces a superior type of extract because it is clearer and less colored. However, cigarette smoke contains certain volatile esters, aldehydes, ketones, and other organic substances, parts of which are undoubtedly soluble in organic solvents; these organic substances, which might act as allergens, would be removed if the tobacco mass were first defatted by an organic solvent.

Therefore, two types of mixed tobacco extracts were prepared. One was defatted by washing and decanting with toluene before the aqueous extraction, and the other was treated with no organic solvent before the aqueous extraction. The tobacco mass was then extracted in buffered saline (pH 7.0) for two days. After filtration, the extract was dialyzed against buffered saline for forty-eight hours and then concentrated by allowing the extract, in cellophane sausage casing, to hang in front of an electric fan. After Seitz filtration and sterility tests, each extract was standardized on a protein nitrogen basis according to the method of Stull and Cooke. The tobacco extracts used for testing were in 1,000 units phosphotungstic-acid-precipitable nitrogen strength.

*Original wheal—negative reaction—0.2 to 0.3 cm. wheal. Slight reaction—0.4 to 0.6 cm. wheal. Moderate reaction—1 to 1.5 cm. wheal. Marked reaction—2 plus cm. with pseudopodia.

Before skin testing, each subject was thoroughly interrogated as to any personal and familial history of allergy and was questioned specifically about peripheral vascular symptoms and pertinent smoking habits. Each subject was carefully questioned as to the following specific points:

On Allergy—Personal or familial occurrence of seasonal coryza, bronchial asthma, urticaria, food sensitivity, and drug sensitivity.

On Peripheral Vascular Disease—Evidence of numbness of extremities and fainting spells.

On Symptoms Directly Related in Time to Tobacco Smoking.—Coughing, dizziness, headaches, effect on appetite, and cold, numbness, and tingling of the extremities.

Eighty healthy adults who had been skin tested with tobacco extract were studied in the New York University Research Service at Goldwater Memorial Hospital. The vascular responses of these persons were tested by automatic recording of surface temperature and by large-limb venous occlusion plethysmograph in a room with constant temperature and humidity under environmental conditions set at 20° and 25° C. with 55 per cent humidity. Measurements were taken before the subjects smoked a cigarette, and at 2-, 6-, and 20-minute intervals following the onset of the smoking procedure. Changes in blood flow greater than 2 c.c. per 100 ml. of tissue per minute and changes in surface temperature of at least 2° C. were considered significant. Ballistocardiograms and electrocardiograms were also obtained on these volunteers, and they were interpreted by the standard criteria. The cardiovascular measurements were recorded before, during, and after the smoking of special cigarettes composed of a mixture of tobaccos from all the above-mentioned commercial cigarettes.

RESULTS

Cutaneous Reactions to Tobacco Extracts.—Six hundred forty-one healthy adult volunteers, both smokers and nonsmokers, were skin tested with five different tobacco extracts. Table I shows the number and percentage of cutaneous reactions, ranging from negative to marked, to Burley, Virginia, Turkish, mixed (not defatted), and mixed tobaccos in a group of 641 adults. Of the persons

TABLE I. PERCENTAGE OF VOLUNTEERS TESTED BY REACTIONS TO EACH OF FIVE TOBACCO EXTRACTS BY SMOKING HISTORY

	SMOKERS					NONSMOKERS				
EXTRACT	TOTAL	NEGATIVE	SLIGHT	MODERATE	MARKED	TOTAL	NEGATIVE	SLIGHT	MODERATE	MARKED
Burley	379	71.5	15.3	5.0	8.2	261	73.6	15.7	5.4	5.4
Virginia	378	70.4	14.3	6.1	9.3	261	73.6	14.6	3.8	8.0
Turkish	379	71.0	15.6	4.0	9.5	261	75.5	12.3	3.8	8.4
Mixed	341	68.3	12.6	5.3	13.8	234	73.5	14.5	2.1	9.8
Mixed (defatted)	340	70.3	13.5	7.4	8.8	232	73.7	13.8	5.6	6.9
Total*	380	63.2	12.4	6.1	18.4	261	65.5	15.3	5.4	13.8

*Each person classified by most severe reaction to any of five extracts. No statistical difference between smokers and nonsmokers.

tested, 18.4 per cent of the smokers showed markedly positive reactions to one or more of the tobacco extracts, as compared with 13.8 per cent of the nonsmokers. The difference between the percentages of smokers and nonsmokers reacting to each extract was not found to be statistically significant. Table I also indicates that there were more reactions to the mixed brand of tobacco extract that was not defatted. The defatting process would remove an appreciable quantity of esters, aldehydes, and other organic compounds ordinarily thought to be volatilized in the smoke of the cigarette. This mixed extract also contains, besides tobacco, unrelated allergens and irritating chemicals. It was noted that in the positively reacting group, some persons reacted to only one of the extracts, some to two, some to three, some to four, and some to all five extracts. By the skin-test criterion, a person who was skin sensitive to tobacco could therefore be sensitive to only one tobacco and the tobacco product of one provenance and not be sensitive to any other type.

No attempt was made to classify the smokers as occasional, moderate, or heavy smokers, as it was found that such classification would be impossible or would more likely be misleading than instructive. Many of the subjects smoked different amounts at different times, some ranging from one or two cigarettes daily during certain periods of their lives to a pack or more at other periods. If it had been possible for us to make an accurate quantitative breakdown of smoking habits, this might have been informative. We do not consider it essential, however, since the incidence of positive skin tests was not significantly greater in the entire group of smokers as compared with entire group of nonsmokers.

Sex and age did not seem to play any role in the incidence of the reactions to tobacco in the various groups tested.

Relationship of Peripheral Vascular Symptoms to Cutaneous Tobacco Reactions.—Table II sets forth the percentage of general vascular symptoms in a group of 377 smokers tested with Virginia tobacco extract. It can be seen from these statistics that peripheral vascular symptoms were approximately twice as

TABLE II. PERCENTAGE OF VOLUNTEERS WITH HISTORY OF PERIPHERAL VASCULAR SYMPTOMS AND REACTIONS TO THE VIRGINIA TOBACCO EXTRACT

TOTAL NUMBER	SKIN TEST	NUMBNESS OF EXTREMITIES (PER CENT)	MUSCULAR CRAMPS OF EXTREMITIES (PER CENT)	COLD EXTREMITIES (PER CENT)	TINGLING OF EXTREMITIES (PER CENT)
Smokers (377)	Positive (57)	28	26	30	30
	Negative (320)	14	11	13	15
Nonsmokers (260)	Positive (31)	16	19	16	10
	Negative (229)	14	13	13	11

A significant association was found between smokers with positive skin reactions to tobacco and:

Numbness of the extremities—$\times 2 = 6.0$; $n = 1$; $P = <0.02$.

Cold extremities—$\times 2 = 8.6$; $n = 1$; $P = <0.01$.

Muscular cramps of the extremities—$\times 2 = 11$; $n = 1$; $P = <0.01$.

Tingling of the extremities—$\times 2 = 6.6$; $n = 1$; $P = <0.01$.

The above results tend to show that among the nonsmokers there appeared to be no association of any of the four symptoms with the positive skin tests to tobacco.

common in the smokers with positive skin tests than in the smokers with negative skin reactions. In the nonsmokers, the incidence of peripheral vascular symptoms was about the same, whether the skin-test reaction to tobacco was positive or negative.

Relationship of the Allergic State to Cutaneous Tobacco Reactions.—Table III lists the percentage of volunteers with manifestations of personal allergy and the reactions to the Virginia tobacco extract. This table shows the increased incidence of positive skin reactions to tobacco among the allergic persons, both smokers and nonsmokers. Over twice as many persons with positive reactions reported having seasonal rhinitis, bronchial asthma, or urticaria as compared with the subjects with negative skin reactions. In the group of smokers reporting a history of allergic manifestations, 53.4 per cent were found to react in a positive manner to one or more of the tobacco extracts. In contrast, only 18.7 per cent of the normal nonallergic smokers reacted to the tobacco extract. Among the nonsmokers, 40.9 per cent of the allergic persons were found to give positive reactions, and 14.7 per cent of the normal nonsmokers reacted positively to the tobacco extract. This finding would seem to indicate that the reaction to the tobacco extract may be evidence of multiple sensitization characteristic of the allergic state. A skin sensitivity to tobacco could well be more readily acquired by the generally allergic person than by the generally less allergic one on intimate or casual contact with tobacco smoke and tobacco products.

TABLE III. PERCENTAGE OF VOLUNTEERS WITH MANIFESTATIONS OF PERSONAL ALLERGY AND REACTIONS TO THE VIRGINIA TOBACCO EXTRACT

TOTAL NUMBER	SKIN TEST	SEASONAL RHINITIS (PER CENT)	BRONCHIAL ASTHMA (PER CENT)	URTICARIA (PER CENT)	OTHERS* (PER CENT)
Smokers (377)	Positive (57)	7	18	26	37
	Negative (320)	4	4	5	18
Nonsmokers (260)	Positive (31)	19	19	16	13
	Negative (229)	7	4	7	16

*Food sensitivity, drug sensitivity, and rashes.

Among smokers there was no significant association between positive skin tests to tobacco and seasonal rhinitis.

Among smokers a significant association was found between positive skin tests to tobacco and:

Bronchial asthma—$\times 2 = 12.9$; $P = <0.01$.

Urticaria—$\times 2 = 26.2$; $P = <0.01$.

Among nonsmokers no significant association was noted between the positive skin test reaction to tobacco and the manifestations of personal allergy.

Symptoms Related to Cigarette Smoking and the Cutaneous Reactions to Virginia Tobacco Extract.—In Table IV it is noted that 24 per cent of the smokers with positive tests to tobacco gave evidence of coughing spells directly related to the act of smoking, as compared with 12 per cent of the smokers who had negative skin reactions. Symptoms of cold, numbness, and tingling of the extremities associated with smoking were reported by 8 per cent of the smokers with positive tests and by 2 per cent of those with negative reactions. There

was no apparent difference in incidence of dizziness, headache, and effect on appetite among the smoking group, whether they reacted in a positive or negative manner to the tobacco extract.

TABLE IV. PERCENTAGE OF SMOKERS WITH HISTORY OF TOBACCO SYMPTOMS AND REACTIONS TO THE VIRGINIA TOBACCO EXTRACT

TOTAL NUMBER	SKIN TEST	COUGHING (PER CENT)	DIZZINESS (PER CENT)	HEADACHES (PER CENT)	EFFECT ON APPETITE (PER CENT)	COLD, NUMBNESS, AND TINGLING OF EXTREMITIES (PER CENT)
57	Positive	24	10	6	8	8
320	Negative	12	10	2	6	2

A significant association was found between the positive skin test to tobacco and the incidence of:

Coughing when smoking—$\times 2 = 5.2$; $P = <0.02$.

Cold, numbness, and tingling of the extremities—$\times 2 = 5.5$; $P = <0.02$.

Relationship of Allergy, Peripheral Vascular Symptoms, and Cutaneous Reactions to the Tobacco Extracts.—Table V is presented in an attempt to correlate allergic manifestations, peripheral vascular symptoms, and the skin reactions to tobacco extract. It was found that 33 per cent of nintey-six smokers with positive skin tests to tobacco gave a history of allergic manifestations and reported having symptoms of peripheral vascular disturbances. Only 10 per cent of 291 smokers with negative skin tests gave evidence of both allergy and peripheral vascular symptoms. Among the nonsmokers, whether they gave positive or negative reactions, no significant difference in incidence of allergy and vascular symptoms was noted.

TABLE V. NUMBER OF SMOKERS WITH PERSONAL ALLERGY AND PERIPHERAL VASCULAR SYMPTOMS AND SKIN REACTIONS TO TOBACCO EXTRACTS

	POSITIVE SKIN TEST	NEGATIVE SKIN TEST	TOTAL NUMBER
Positive allergy, positive peripheral vascular symptoms	32 (33%)	30 (10%)	62
Positive allergy, negative peripheral vascular symptoms	17 (18%)	71 (25%)	88
Negative allergy, positive peripheral vascular symptoms	13 (14%)	35 (12%)	48
Negative allergy, negative peripheral vascular symptoms	34 (35%)	155 (53%)	189
Total number	96	291	387

$\times 2 = 30.0$; $n = 3$; $P = <0.001$.

Vascular Responses to Smoking and Cutaneous Reactions to Tobacco Extracts.—Tables VI and VII list the skin temperature and blood flow changes in eighty healthy adults, with their skin-test reactions to tobacco extracts. From this study, it was learned that blood flow and skin temperature changes appear to be independent, since few persons gave reactions to both of these vascular responses. With respect to changes in blood flow, no significant difference was found between those giving positive and those giving negative skin tests to tobacco extracts. On the other hand, skin temperature changes

were noted in 28 per cent of the smokers giving positive skin-test reactions and in only 4 per cent of those giving negative reactions. The difference is statistically significant.

TABLE VI. CHANGES IN SKIN TEMPERATURE AND BLOOD FLOW ON EIGHTY SMOKERS AND SKIN REACTIONS TO TOBACCO EXTRACTS

	BURLEY		VIRGINIA		TURKISH		MIXED		MIXED DEFATTED	
	POSITIVE	NEGATIVE	POSITIVE	NEGATIVE	POSITIVE	NEGATIVE	POSITIVE	NEGATIVE	POSITIVE	NEGATIVE
T+ B+	1		1		1		1		1	
T+ B–	6	4	5	5	5	5	4	6	5	5
T– B+	9	11	7	13	7	13	9	11	7	13
T– B–	8	41	9	40	10	39	12	37	8	41
Total	24	56	22	58	23	57	26	54	21	59

T = Skin temperature. + = Positive.
B = Blood flow. – = Negative.

The electrocardiographic and ballistocardiographic recordings were not included in this discussion, since so few volunteers presented any significant changes and since skin tests with tobacco extracts did not appear to be correlated with the results obtained.

TABLE VII

	POSITIVE REACTORS	NEGATIVE REACTORS
T+ B+	1	0
T+ B–	8	2
T– B–	11	9
T– B–	12	37
Total	32	48

T = Skin temperature. + = Positive.
B = Blood flow. – = Negative.

Positive reactors to the tobacco extracts are more likely to show changes in skin temperature after smoking. $\times 2 = 2.6$; $n = 1$; $P = <0.01$.

Cutaneous Reactions to Tobacco Among Allergic and Nonallergic Children.—A total of 294 children between the ages of 1 and 5 years were skin tested with the five tobacco extracts. Of this total, 11.5 per cent were found to react in a markedly positive manner to one or more of the tobacco extracts. Thorough questioning of the parents of 269 of the children revealed no evidence of any allergic manifestations. Therefore, these children were considered normal for purposes of this study. Of these normal children, 6 per cent reacted positively to tobacco on skin testing. Twenty-five of the children were found to be allergic and, of these, 64 per cent gave evidence of skin sensitivity to the tobacco extract on skin testing. None of these children had ever smoked, but they had had the same exposure to tobacco smoke and other tobacco products as other nonsmokers. Practically all of these children were exposed to one or more smokers within the immediate family at home. Here again, as in the adult group, it would seem that the generally allergic disposition of the person renders him very receptive to the development of multiple skin sensitization, including

sensitization to tobacco, on casual or intimate exposure to the allergen. There was no difference in the incidence of positive skin reactions to tobacco in the various age groups. Female subjects reacted in the same proportions as male subjects.

SUMMARY AND INFERENCES

A group of 641 healthy volunteers were questioned concerning personal allergies, smoking history, symptoms presumed to be connected with smoking, and peripheral vascular disturbances.

These persons were skin tested by the intradermal method with five different tobacco extracts: Burley, Virginia, Turkish, and mixed tobaccos. The mixed tobacco extract was made by combining tobaccos from six popular cigarettes.

It was found that about 15 per cent of 641 adults reacted in a positive manner to one or more of the tobacco extracts. The per cent reacting was essentially the same in the smokers as in the nonsmokers.

It might have been argued that the heavier smokers, by exposing themselves to more tobacco smoke, became skin reactive to tobacco extracts more frequently. This argument scarcely appears valid, however, in view of our finding that smokers did not react to skin tests with tobacco extracts significantly more often than nonsmokers.

An increased incidence of peripheral vascular symptoms was noted in the smokers with positive reactions to the tobacco, as compared with the smokers who did not react on skin testing with the tobacco.

It might also be argued that excessive smoking could lead to increased incidence of skin-test reactions to tobacco and that the amount of smoking might be an indication of the degree of the patient's anxiety and tension.

In view of the absence of any difference between smokers and nonsmokers in incidence of skin reactions to tobacco, however, it appears extremely unlikely that the degree of anxiety and tension could be a factor in producing skin-test reactions. Moreover, in similar fashion, since the increased smoking could not be proved responsible for increased skin reactions to tobacco extracts, increased anxiety and nervousness could not explain the increased incidence of peripheral vascular symptoms in the smokers with positive skin tests to tobacco as compared to those with negative skin tests.

Volunteers with positive tests to the tobacco extracts reported a history of nontobacco-related allergic manifestations more frequently than the group with negative reactions.

Of the smokers with positive skin reactions to tobacco extracts 33 per cent gave a personal history of allergic manifestations and peripheral vascular symptoms, as compared with 10 per cent of the smokers who did not react to the skin tests with tobacco extracts.

Eighty of these persons were investigated further for circulatory disturbances by means of plethysmographic measurements of blood flow to the lower extremities and automatic recording of surface temperature. These

tests were performed before, during, and after the smoking of a mixed-tobacco cigarette. These experiments indicated that, on smoking the mixed-tobacco cigarette, 23 per cent of the smokers with positive skin tests to tobacco had changes in peripheral circulation indicated by skin temperature as compared with the smokers with negative skin tests, of whom 4 per cent had changes in skin temperature.

In a group of 294 children between the ages of 1 and 5 years tested with the tobacco extracts, 11.5 per cent reacted in a positive manner to one or more of the tobacco extracts. In the small group of allergic children tested, 64 per cent gave evidence of skin sensitivity to the tobacco extracts as compared with 6 per cent of a nonallergic control group.

The close and consistent parallelism between the positive cutaneous reactions to tobacco extract and symptoms of peripheral vascular disturbances, tobacco symptoms, and personal history of allergic manifestations might possibly serve as a clue to the interpretation of the tobacco reactions that we obtained The findings indicate that our positive skin-test reactions to tobacco extracts were based on an allergic (that is, immunologic) mechanism and were associated in more than normal incidence with hypersensitivity to other allergens as well as with fall of peripheral temperature on smoking and with certain clinical peripheral vascular symptoms. It is felt that, although the number of volunteers tested is small, there is a suggestion that the skin test with tobacco may be helpful as a "screening" test to aid in determining the possible importance of tobacco as an etiological factor in certain forms of peripheral vascular symptomatology.

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IMMUNE SERA IN THE STUDY OF FOOD ANTIGENS

I. Fish

Sheldon G. Cohen, M.D.,* Wilkes-Barre, Pa.
With the Technical Assistance of John Saba**

INTRODUCTION

TECHNICAL difficulties experienced in attempting to isolate purified antigenic fractions from foodstuffs have hindered studies of possible biochemical and immunologic identity between food allergens of man and precipitin-producing antigens of experimental animals. Although the reagin of natural allergic man and the serum precipitin of the immunized rabbit differ in physicochemical properties and characteristic biologic actions, they do possess a common denominator of specific reactivity with the same antigen. Thus, any in vitro experimental study involving a soluble food antigen-precipitating antibody system may offer only indirect evidence of the potential role of the particular food as an allergen in man. However, in view of the specificity of action of the in vitro precipitin test, we have attempted to utilize this objective experimental tool in studying some problems related to food allergens that could not be resolved satisfactorily by either direct or indirect intracutaneous tests alone.

The general experimental approach revolved around the ability of immune serum, through its content of precipitating antibodies, to react with specific antigenic counterparts. Antisera from rabbits immunized to fish extracts were thus employed in an in vitro detection of antigenic components of fish-derivative preparations.

EXPERIMENTAL PROCEDURES AND RESULTS

Male rabbits, varying in weight between 2.5 and 3 kilograms, were utilized in active immunization procedures for the production of specific antisera to fish antigens. The immunizing preparations consisted of fluid glycerosaline extracts† of cod (genus *Gadus*) and halibut (genus *Hippoglossus*). Each rabbit received a total of 12 mg. of protein nitrogen of either halibut or cod fish extract through a series of twelve equally divided doses given by intravenous injections over a period of twenty-one days. Antisera were prepared from blood taken by cardiac puncture seven days after the last injection.

From the Department of Biology, Wilkes College.

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*Associate Professor of Biological Research.

**Student Laboratory Assistant.

†Prepared and supplied through the courtesy of Margaret Strauss, Director of the Allergy Laboratory, University Hospital, New York University–Bellevue Medical Center.

Pooled sera showing equivalent high titers to cod extract and similarly pooled antisera to halibut extract were subsequently utilized in precipitin test procedures.

Qualitative antigenic analyses of fish extracts and fish-derivative preparations were carried out by precipitin test techniques in agar-filled Petri dishes, according to the double diffusion method described by Ouchterlony.[1] The soluble fish preparation and the antiserum to fish extract were placed in oppositely located wells within the agar to allow for their interreaction while diffusing through the gel. Since each band of precipitate represented an independent antigen-antibody system, these reactions permitted the resolution of reactants into individual components. Reactions of identity and those of nonidentity between individual antigenic constituents of soluble fish preparations were determined by allowing each to diffuse from a parallel position within the agar against a common reference antiserum to the fish extract.

The antisera employed in all subsequently described experiments possessed the following characteristics: (1) antiserum to cod contained six immunologically distinct reactive antibody components, and (2) antiserum to halibut contained five such components.

Fish-Liver Oils.—Commercially available cod-liver oils and halibut-liver oils, utilized as sources of vitamins A and D, were studied for possible fish antigen content. Random lots of cod-liver oil and Haliver oil from different pharmaceutical houses were obtained on the open market, and each was utilized in two different types of experiments.

Series of rabbits were injected with either one of the five cod-liver oil preparations or one of the two Haliver oil preparations. Each animal received 1 c.c. of the oil twice weekly via alternating intramuscular and intraperitoneal injections. After the third week, and at weekly intervals thereafter, each rabbit was bled from the central artery of the ear and the serum was separated and studied for possible antibody content to the homologous fish extract. These semiweekly injections and weekly bleedings were continued over a sixteen-week period. In vitro tests were carried out with each weekly sample of rabbit sera and a fish extract of proved antigenicity (halibut extract containing six distinct antigens and codfish extract with five individual antigenic constituents). In no instance was the development of an antigen-antibody precipitate noted.

An oil-in-water emulsion was prepared from each lot of cod-liver oil and Haliver oil in amounts equal to those employed in immunization procedures. After repeated agitation over a three- to four-day period, the oil and water were allowed to separate in layers and were individually collected. The aqueous fraction of each preparation was employed in agar-gel diffusion precipitin tests with antisera to fish extracts. The results are shown in Table I.

Fish Emanations.—

Boiled fish: Small pieces of fresh raw cod were placed in a distilling flask containing water, and the contents of the flask were heated to boiling temperatures. The resultant steam was led off directly through glass tubing into a water-cooled condensor, and the distillate was collected. The same procedure was repeated for raw halibut.

Fried fish: The above-described apparatus was modified to collect the distillates of fish-in-oil fumes of fresh raw cod and of halibut frying in hydrogenated cooking fats. Oil-in-water emulsions of the distillate were prepared and thoroughly agitated, and the aqueous fraction was separated and collected.

Raw fish odors: Raw ground cod was placed in refrigerated empty sterile filtering flasks. Rubber stoppers were used to close off the openings at the side arms and mouths of the flasks. At periodic intervals over five days the flasks

TABLE I

AQUEOUS EXTRACT	ANTISERUM TO:	NUMBER OF PRECIPITIN BANDS
Cod-liver oil	Codfish	
Brand A	Codfish	1-2
Brand B	Codfish	1-3
Brand C	Codfish	0
Brand D	Codfish	2-5
Brand E	Codfish	0
Halibut-liver oil	Halibut	
Brand A	Halibut	2
Brand B	Halibut	0-1

were removed from the refrigerator and the air within the flask was aspirated by means of a vacuum pump through the side arms. The fish odor-laden air was then led off through tubing and bubbled directly into the buffered saline contents of sterile vials. Similar procedures were carried out with fresh ground raw halibut.

Precipitin tests: The steam distillates, aqueous extracts of the oil distillates, and saline solutions of raw and decomposing fish emanations of both cod and halibut were employed in agar-gel diffusion tests with high-titer antisera to cod and halibut, respectively. In no instance was a visible band of precipitate noted within the agar.

TABLE II

TEST PHARMACEUTICAL	ANTISERUM TO:	NUMBER OF PRECIPITIN BANDS
Protamine sulfate	Cod	1-2
Protamine sulfate	Halibut	2
Sodium morrhuate	Cod	5
Sodium morrhuate	Halibut	5

Fish-Derivative Pharmacologic Agents.—Solutions of protamine and sodium morrhuate injectable pharmaceutical preparations obtained on the open market were employed in agar-gel diffusion precipitin tests with antisera to cod and to halibut. The results are shown in Table II.

Fish Roe.—An extract was prepared from the roe of yellow pike (*Stizostedion vitreum*), utilizing buffered saline according to standard methods of extraction. Antisera to fish roe were prepared according to the immunization techniques previously described for the production of fish antisera. Agar-gel

diffusion precipitin tests were carried out with fish roe extract, homologous antisera, and the antigenic preparations of chicken egg yolk and egg white and their antisera, the preparation and properties of which were described in a previous report.[2]

The interreaction of antiserum to fish roe and homologous fish roe extract resulted in the production of two bands of precipitate. However, in tests employing (1) antiserum to fish roe with chicken egg white and egg yolk and (2) fish roe extract with the antisera to chicken egg white and egg yolk, no precipitin bands could be noted.

Fish Extracts.—Randomly selected samples of cod and halibut fluid extracts prepared in different laboratories for purposes of skin testing were employed in agar-gel diffusion tests with the antisera to cod and to halibut. The results are summarized in Table III.

TABLE III

ANTISERUM TO:	EXTRACT	NUMBER OF PRECIPITIN BANDS			
		A	B	C	D
Cod	Cod	5-6	0-3	3-4	4-5
Halibut	Halibut	3-5	0	4	

COMMENT

None of the commercially available lots of cod- and halibut-liver oils employed were found to be capable, by these techniques, of inducing antibody formation to the identifiable antigens of homologous fish-muscle extracts. If the ability of an animal-derived oil to induce precipitating antibody in the rabbit were accepted as the sole requirement of its protein content, one would be tempted to infer that these fish-liver oils were protein-free. By aqueous treatment of the oil, however, sufficient fish protein could be extracted and concentrated to react with specific antibody components of high-titer antifish sera. Even though the total amount present was apparently below the quantitative critical level required for precipitin production, its antigenic potential in animals already sensitized must be considered.

The fact that fish-liver oils may contain protein fragments and carbohydrates and their derivatives is taken into account in the processing of these materials.[3] One of the many variables determining the composition of the final product is the extent of preliminary damage to the native oil-containing fish tissue. Hydrolysis and degradation of certain proteins increase their solubility and dispersibility in oil,[3] and fish oils in contact with decomposing fish proteins can absorb and chemically combine with appreciable quantities of nitrogenous compounds.[4] Since the greater part of such constituents is soluble in oil only in anhydrous form, the greater part of the phosphatides, proteins and protein fragments, resinous and mucilaginous materials, and hydrocarbons may be removed by hydration or by alkali treatment. Thus, the second variable in determining the final composition of the oil product is directly related to the

efficacy of the "degumming process." It is, therefore, understandable that different lots of fish-liver oils can vary widely in both quantitative and qualitative protein content.

Allergic-type reactions to ingested fish-liver oils have been reported.[3-7] Whether all such instances represent true hypersensitivity reactions to constituent fish proteins or to an "oil antigen," or perhaps even an untoward reaction to one of the many variable unidentified organic contaminants, is not apparent. The fact that only trace amounts of fish protein were demonstrated in some lots of cod-liver and Haliver oils may help to interpret the clinical observation of Feinberg[6] that instances of fish-oil allergy apparently occur only in fish-sensitive persons with extreme sensitiveness and that the average fish-sensitive patient may tolerate fish oils in average small doses.

Along with the inconstant presence or absence of fish protein in fish-liver oil, these precipitin tests also demonstrated the variability in number of individual antigens in the final product. Thus, along with a quantitative factor, perhaps another important determinant of allergic reactivity for any fish-liver oil may be the variable presence of that particular protein fraction of fish for which the recipient possesses reagins and clinical sensitivity.

The failure of any of the steam or fried distillates or soluble preparations of the emanations of raw fish to react visibly with specific antisera to the fish suggests that none of these materials contained identifiable antigenic fish fractions. In view of the high molecular weight of fish-protein molecules, it is not surprising that they failed to undergo processes of volatilization after aqueous solution or to be carried off adsorbed to hot oil molecules without prior denaturization or degradation. The question then arises as to what explanation may be placed upon those reported clinical observations in which acute allergic symptoms have appeared in patients merely exposed to the odor of raw or cooked fish. Heretofore, these have been attributed to a volatile fraction of fish antigen.[6-8] Furthermore, according to Tuft,[9] the denaturization of fish through cooking may reduce the skin-test activity and nitrogen content of the allergenic extract, but this may be insufficient to be clinically important. A suitable explanation for this finding, therefore, can be only a matter for speculation. Among suggested possibilities, the following are evident: (1) The products of hydrolysis of the proteins of frying or cooking fish, such as ketones and aldehydes, may be nonspecifically irritating to an allergic respiratory mucosa. (2) Only the undenatured protein molecule will meet the antigenic requirements for specific in vitro precipitin reactions with animal antisera, whereas fragments of the molecule existing as polypeptides may serve as degraded antigens of lesser molecular weight or in some haptenic fashion as specific food allergens in the highly reaginic-sensitive human being.

Protamine sulfate is described as any one of a group of simple proteins or "protein-like" substances occurring in combination with nucleic acid in the sperm of fish. It is a commonly employed pharmacologic agent for antiheparin action and for the conversion of insulin into a long-acting preparation. Apparently, reported local sensitivity reactions to injected protamine[10] and to

salmine of protamine zinc insulin[11] are rare. The possibility that some reactions following insulin therapy may be due to the added protamine has been considered and investigated,[11-12] and it has generally been felt that protamine is devoid of allergenic properties.[6] However, the fact that antigenic fish constituents can be identified by these techniques suggests potential allergenicity in fish-sensitive patients.

Sodium morrhuate, the sodium soap of the fatty acids of cod-liver oil, is employed in aqueous solution as a sclerosing agent for varicose veins. Occasional reactions of the hypersensitivity type, such as urticaria and even shock, have been reported.[13-15] Here again, the identification of component fish antigens suggests their possible allergenic role.

The known components of chicken egg yolk[16] include lipovitellin, livetin, lipovitellenin, phosvitin, vitellin, and vitellenin. Of these, lipovitellin (ichthulin), livetin (thuetin), and phosvitin have been identified in some fish roe.[17] The question then arises as to the possibility of cross-antigenicity between these three biochemically related yolk proteins of diverse species origin. However, by immunologic techniques we have been able to note only two distinct antigenic components for fish roe, and these do not appear to possess any immunologic identity with the demonstrated antigenic fractions of the chicken egg.

The results of these serologic tests point out the wide variation in content of precipitin-producing antigens that may exist between species-identical fish extracts. All were submitted as skin-test-active extracts, and presumably they were prepared by similar laboratory techniques. It has not been determined whether these differences can be related to such factors as minor variations in technique of extraction, the state of the fish sources (for instance, frozen or fresh), the length of time elapsed and the efficiency of preliminary refrigeration before extraction was accomplished, the method and duration of storage, and the use of preservatives in the extract. It is also not apparent whether or not such variations in qualitative antigenic content might explain many of the inconstant results observed in skin tests with fish extracts that often confuse clinical interpretation.

In the evaluation of any hypersensitivity reaction, consideration must always be given to the quantitative factor of antigen content in relationship to the existing degree of clinical sensitivity. Such quantitative variations in antigen content common to different species of fish have been demonstrated by Tuft and Blumstein,[8] utilizing passive transfer neutralization techniques with fish-reaginic sera. However, the results of this study also suggest another factor for consideration, namely, the wide variability in qualitative content of multiple antigenic fractions in fish-derivative preparations. It is possible, therefore, that one important determinant of skin-test activity of a fish extract or clinical allergic reactivity for a food or pharmaceutical of fish origin may be the variable presence of that particular protein fraction of fish for which the recipient possesses reagins and clinical sensitivity, respectively. Proof of this, however, must await the results of clinical correlation and a clearer understanding of the exact relationships of precipitin-producing antigens to food allergens. The

importance of these unresolved points as subjects for further investigation is self-evident. Such questions are, therefore, under current consideration and study by the Committee on Food Allergy of the Research Council.

SUMMARY

1. Sera from specifically immunized rabbits were employed in agar-gel diffusion precipitin test techniques in the qualitative antigenic analyses of fish extracts and fish-derivative preparations.

2. Fish antigens were demonstrated in some samples of cod- and halibut-liver oils, with wide variations in components existing among different lots of these materials.

3. Antigenic fish constituents could not be detected in the emanations of raw, fried, or boiled cod and halibut.

4. Fish antigens were identified in protamine and sodium morrhuate.

5. The antigenic components of fish roe did not demonstrate cross-antigenicity with egg proteins of chicken origin.

6. A wide qualitative variation among individual component antigens of fish muscle was demonstrated in homologous species skin-testing extracts of different laboratory sources.

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INHIBITION OF ANAPHYLAXIS IN THE GUINEA PIG BY LOCAL STREPTOCOCCAL INFECTION

The Influence of Adrenalectomy

L. N. Meleyco, M.D., Jacob J. Pruzansky, Ph.D., Samuel M. Feinberg, M.D., and S. Zwi Kantor, M.D.
Chicago, Ill.

IN MAN, partial or complete remission from allergic symptoms has been observed in acute infection and in other conditions which have been classified as "stress." The manifestations of "stress" are currently considered to be mediated through the pituitary and adrenal glands. The effectiveness of ACTH and corticosteroids in the management of various allergic conditions suggests that the observed reduction in hypersensitivity during infection may be due to stimulation of the pituitary and adrenal glands.

One of the procedures developed in our laboratory for the inhibition of anaphylaxis in the guinea pig is the production of a local streptococcal abscess in the axilla. The method was suggested by the observation that a sensitized but otherwise untreated guinea pig was occasionally asymptomatic or exhibited only minor symptoms when subjected to anaphylactic challenge. Investigation revealed that there was a correlation in individual animals between reduced hypersensitivity and the presence of a local, naturally occurring abscess from which group C hemolytic streptococci could be isolated. Experimental production of an abscess with a pure culture of isolated streptococci also resulted in a refractoriness to anaphylaxis. This provided the means for an investigation of the role of the adrenal glands in the inhibition of hypersensitivity in the guinea pig. Although a study of the effect of bilateral adrenalectomy in the inhibition of anaphylaxis by sodium ribonucleate has been reported previously by Benaim and associates,[1] it was felt that the influence of experimental infection may be more closely related to the clinical problem.

METHODS

Anaphylaxis.—The method of Feinberg and Malkiel[2] has been used with minor modifications. Guinea pigs were sensitized passively by the intravenous injection of 31 mcg. of guinea pig antiovalbumin per 100 grams of body weight. Forty-eight hours later the animals were exposed in a chamber to an aerosol of

From the Allergy Research Laboratory, Department of Medicine, Northwestern University Medical School.

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0.2 per cent crystallized ovalbumin. The time necessary for the development of end-point symptoms was recorded as a measure of the intensity of reaction. Sensitized but otherwise untreated animals developed a readily distinguishable syndrome of spasmodic breathing and/or coughing in 60 to 120 seconds. The maximum period of exposure was 600 seconds, since it had been shown that longer exposure consistently failed to elicit end-point symptoms in refractory animals. Guinea pigs remaining in the box for 300 seconds or longer without developing the described symptoms were considered significantly protected.

Some animals were also exposed to an aerosol of a solution of histamine dihydrochloride. An aerosol of 0.2 mg. of the free base per milliliter produced end-point symptoms, indistinguishable from those observed in anaphylaxis, in 60 to 120 seconds.

Production of Streptococcal Abscess.—Pure cultures of group C streptococci were obtained from naturally occurring local infections of guinea pigs and were maintained on blood agar slants. Eighteen-hour broth subcultures were injected into the axillae of guinea pigs. Induration was usually noted in two days, and a fluctuant abscess developed within four to seven days. Drainage often occurred after variable periods. The development of refractoriness was not uniform and depended on the culture and the length of time elapsing between inoculation and challenge. Best results were obtained in animals infected at least eight days prior to challenge and in which complete drainage had not occurred during that period.

Adrenalectomy.—The procedure of Benaim and colleagues[1] was followed. This report confirms the previous findings of a high mortality rate following bilateral adrenalectomy and a short period of survival after removal of the second adrenal. For this reason, it was necessary to infect experimental animals soon after the removal of the right adrenal but several days before removal of the left adrenal. The animals were sensitized just before the second operation and challenged forty-eight hours later. One might argue that this procedure complicated the interpretation of results, since high levels of adrenal steroids may have been built up before the removal of the left adrenal and may have remained in the circulation during sensitization and challenge. The high rate of clearance of adrenal steroids, the relatively high dosages of injected steroids necessary to prevent normal anaphylactic response, and the short survival period of guinea pigs following bilateral adrenalectomy suggest that there was little residual circulating steroid.

RESULTS

The animals were divided into four groups: (1) infected only; (2) infected and adrenalectomized; (3) adrenalectomized only; (4) not infected or adrenalectomized. They were all sensitized forty-eight hours prior to anaphylactic challenge. Some guinea pigs of the second group were also challenged with an aerosol of histamine two hours subsequent to challenge with antigen. The results of these experiments are shown in Table I. These data represent but a small fraction of the total number of animals involved. A large number

of animals were lost during the operative procedure. In addition, the results of some of the complete experiments had to be discarded because of the failure of the infection to inhibit anaphylaxis in some of the unoperated control animals. Infected animals developed a significant refractoriness to anaphylaxis which was almost identical in magnitude in both the adrenalectomized and unoperated groups. The adrenalectomy alone did not significantly affect passively induced hypersensitivity. In contrast to the inhibition of the bronchospasm in infected and adrenalectomized animals, the responsiveness to histamine aerosol was undiminished.

TABLE I. EFFECT OF ADRENALECTOMY ON INHIBITION OF ANAPHYLAXIS BY STREPTOCOCCAL INFECTION

	INFECTION ONLY	INFECTION AND ADRENALECTOMY	ADRENALECTOMY ONLY	NOT INFECTED AND NOT ADRENALECTOMIZED
Number of animals	20	22	10	20
Anaphylaxis time (seconds)	462 ± 159	444 ± 160	98 ± 21	71 ± 6
Histamine time (seconds)	90 ± 19	66 ± 5	----	85 ± 13

DISCUSSION

The adrenal glands appear to play no role in the observed inhibition of anaphylaxis in guinea pigs infected locally with a group C hemolytic streptococcus. Whether the experimental infection is a true model for the reduced clinical sensitivity resulting from infection or other nonspecific effects cannot be determined from these data. Clinically, remission may develop very rapidly in contrast to the lengthy lag period observed in experimental animals. This difference may not be as significant as it appears at first sight, since a number of animals that had complete drainage of the original abscess and were infected a second time were refractory when tested only five days after reinfection. The rapid response in clinical infection may, therefore, have been due to previous infections with the same organism.

The eight- to eleven-day period necessary for inhibition after a first infection and the reduction to five days, or possibly less, after reinfection suggest that antibody production may be a factor. It is impossible at present, however, to specify the exact mechanism by which clinical or experimental infection alters hypersensitivity.

Surgical shock alone did not significantly affect anaphylactic responsiveness in contrast to clinical observations of remission from allergy following major surgery. The bronchospasm produced by histamine aerosol was unaffected by these procedures, indicating normal tissue reactivity.

SUMMARY

1. Guinea pigs infected locally with a group C streptococcus developed refractoriness to anaphylaxis after a suitable induction period. Bilateral adrenalectomy of guinea pigs after infection but forty-eight hours before challenge had no effect on the manifestation of hyporesponsiveness.

2. Surgical trauma did not reduce passively induced hypersensitivity.

3. Infection in adrenalectomized animals did not alter the histamine sensitivity of the tissues.

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American Academy of Allergy

FIFTEENTH ANNUAL MEETING
OF
THE AMERICAN ACADEMY OF ALLERGY
CHICAGO, ILLINOIS
FEB. 9, 10, AND 11, 1959

SCIENTIFIC PROGRAM

Monday, Feb. 9, 1959

Morning Session, 9 A.M.

IRVING W. SCHILLER, M.D., *Chairman*
MILTON M. MOSKO, M.D., *Co-Chairman*

1. **Cutaneous Reactions to Autologous and Non-Autologous Plasma** (Abstract No. 11).
   Samuel O. Freedman, M.D., J. Q. Bliss, M.D., J. L. Hutchison, M.D., and A. S. V. Burgen, M.D., Montreal, Quebec.
2. **Purification of Antibodies: Studies of Antibodies Eluted From Dextran-Type II Pneumococcal Antiserum Precipitates by Treatment With Dextranase** (Abstract No. 27).
   Oscar Swineford, Jr., M.D., Rudolf Hoene, M.D., Ph.D., and Susan Quelch, B.S., Charlottesville, Va.
3. **Immune Sera in the Study of Food Antigens. I. Fish. II. Crustaceans** (Abstract No. 4).
   Sheldon G. Cohen, M.D., Wilkes-Barre, Pa.
4. **The Effect of Heat Processing and Gamma Radiation on the Anaphylactogenic Properties of Milk** (Abstract No. 16).
   H. F. Kraybill, Ph.D., Denver, Colo.
5. **Studies of the Antigenic Relationship of Insect Extracts by Reagin Neutralization. Evidence for Non-Specific Inactivation of Reagin** (Abstract No. 9).
   Jacob J. Pruzansky, Ph.D., Samuel M. Feinberg, M.D., Arthur Kaminker, M.D., and Leo N. Meleyco, M.D., Chicago, Ill.
   ADDRESS: An Inquiry Into the Pathogenesis of Allergic Reactions of the Anaphylactic Type. Frederick G. Germuth, Jr., M.D. (By Invitation). Pathologist in Chief, Director of Laboratories, Charlotte Memorial Hospital, Charlotte, North Carolina.
6. **Parallel Volumetric and Gravimetric Pollen Counts. I. The Variabilities and Inconsistencies of a Conversion Factor** (Abstract No. 25).
   George I. Blumstein, M.D., and Jay Spiegelman, M.D., Philadelphia, Pa.
7. **The Antigenicity of Estrone-Protein Conjugates** (Abstract No. 23).
   Bram Rose, M.D., L. Goodfriend, and A. H. Sehon, Ph.D., Montreal, Quebec.
8. **An Immuno-Chemical Method for the Determination of the Serum Level of Insulin** (Abstract No. 7).
   Richard J. Feinberg, M.D., Chicago, Ill.

Afternoon Session, 2 P.M.

T. L. SQUIER, M.D., *Chairman*
S. F. HAMPTON, M.D., *Co-Chairman*

9. **Sedimentation Studies of Skin Sensitizing Antibody** (Abstract No. 15).
Ernest M. Heimlich, M.D., W. E. Vannier, M.D., Ph.D., and Dan H. Campbell, Ph.D., Pasadena, Calif.

10. **Studies on the Simultaneous Inhibition of Anaphylatoxin Formation and Complement Fixation** (Abstract No. 20).
Abraham G. Osler, Ph.D., Betsy M. Hill, Sc.M., Harry G. Randall, B.A., Zoltan Ovary, M.D., and Eugene Rodriguez, B.S., Baltimore, Md.

11. **The Effect of Hyposensitization Therapy on the Hemagglutinating Antibodies in Ragweed Sensitive Patients** (Abstract No. 1).
Carl E. Arbesman, M.D., S. Zwi Kantor, M.D., Robert B. Beede, M.D., and Noel R. Rose, M.D., Buffalo, N. Y.

12. **Skin Sensitizing Activity of Human Serums and Serum Fractions** (Abstract No. 18).
Frederick Aladjem, Ph.D., Walter R. MacLaren, M.D., Robert R. Jaross, B.S., and Padmisini K. Ayengar, Ph.D., Los Angeles, Calif.
ADDRESS: Genetics and Medicine. Barton Childs, M.D. (By Invitation). Associate Professor of Pediatrics, Johns Hopkins University Medical School, Baltimore, Md.

13. **On Modification of Penicillin Antigenicity** (Abstract No. 3).
George H. Berryman, M.D., and Elmer W. Fisherman, M.D., Chicago, Ill.

14. **Anaphylactic Reactions in the Skin of the Guinea Pig With Horse, Sheep and Chicken Antibodies** (Abstract No. 21).
Zoltan Ovary, M.D., Baltimore, Md.

15. **In Vitro Production of Rabbit Antibody** (Abstract No. 31).
J. H. Vaughan, M.D., R. W. Dutton, M. George, and A. H. Dutton, M.D., Rochester, N. Y.

16. **Effect of Specific Desensitization on In Vitro Histamine, Release From Allergic Blood** (Abstract No. 29).
Paul P. Van Arsdel, Jr., M.D., and Elliott Middleton, Jr., M.D., Seattle, Wash.

Tuesday, Feb. 10, 1959

Morning Session, 9 A.M.

JEROME GLASER, M.D., *Chairman*
HARRY L. MUELLER, M.D., *Co-Chairman*

17. **The Relationship Between Bronchiolitis and Childhood Asthma** (Abstract No. 14).
Heinz J. Wittig, M.D., N. J. Cranford, and Jerome Glaser, M.D., Rochester, N. Y.

18. **Food Allergy: A Study of 150 "Allergic" Children** (Abstract No. 12).
Stanley S. Freedman, M.D., William Sellars, M.D., Providence, R. I.

19. **Evidence of Allergy in Patients With Bronchial Asthma Due to Chronic Cystic Fibrosis** (Abstract No. 30).
Thomas E. Van Metre, Jr., M.D., Robert E. Cooke, M.D., Edward L. Gibson, M.D., and Walter L. Winkenwerder, M.D., Baltimore, Md.

20. **Abnormal Sweat Electrolytes in Patients With Allergies** (Abstract No. 17).
Gilbert Lanoff, M.D., Oliver Crawford, M.D., Marvin Smoller, B.S., and David Yi-Yung Hsia, M.D., Chicago, Ill.

21. **Pulmonary Function in Normal and Asthmatic Children** (Abstract No. 2).
I. Leonard Bernstein, M.D., Louis Kreindler, M.D., Joseph E. Ghory, M.D., Moshe Gerond, M.D., and Ronald G. Fragge, M.D., Cincinnati, Ohio.

ADDRESS: Emerging Patterns of Organic Metabolism in Allergy. Lytt I. Gardner, M.D. (By Invitation). Professor of Pediatrics, State University of New York, Upstate Medical Center, Syracuse, New York.

22. **The Spatial Vectorcardiogram in Bronchial Asthma—A Clinical and Cardiographic Study** (Abstract No. 5).
Leo H. Criep, M.D., and Marvin Silverblatt, M.D., Pittsburgh, Pa.

23. **Continuous Corticosteroid Therapy in Asthma** (Abstract No. 33).
Walter L. Winkenwerder, M.D., and Leslie N. Gay, M.D., Baltimore, Md.

ADDRESS: More Stress on Education in Allergy. Merle W. Moore, M.D., Portland, Oregon. President, American College of Allergists.

12:15 P.M. President's Luncheon

Afternoon Session, 2:30 P.M.

LEON UNGER, M.D., *Chairman*
E. L. KEENEY, M.D., *Co-Chairman*

24. **Metabolic Studies on Asthmatic Patients Treated With Newer Steroids** (Abstract No. 24).
Eleanor McGarry, M.D., Eleanor Venning, M.D., and Bram Rose, M.D., Montreal, Quebec.

25. **Rate of Recovery of Normal Adrenal Cortical Function After Dexamethasone Therapy** (Abstract No. 13).
Henry T. Friedman, M.D., and John F. Murray, M.D., Beverly Hills, Calif.

ADDRESS: Hormones and Connective Tissue. Rachmiel Levine, M.D. (By Invitation). Chairman, Department of Medicine, Michael Reese Hospital, Professor of Medicine, Chicago Medical School, Chicago, Illinois.

4 P.M. Annual Business Meeting of the American Academy of Allergy. Committee on Drugs Report.

Wednesday, February 11, 1959

Morning Session, 9 A.M.

FRANCIS C. LOWELL, M.D., *Chairman*
DAVID W. TALMAGE, M.D., *Co-Chairman*

26. **Fever as a Manifestation of an Experimental Allergy** (Abstract No. 6).
Richard S. Farr, M.D., Pittsburgh, Pa.

27. **Inhibition of Anaphylaxis in the Guinea Pig by Local Streptococcal Infection. The Influence of Adrenalectomy** (Abstract No. 8).
Leo N. Meleyco, M.D., Jacob J. Pruzansky, Ph.D., Samuel M. Feinberg, M.D., and Samuel Z. Kantor, M.D., Chicago, Ill.

28. **Does the Allergic Diathesis Influence Malignancy?** (Abstract No. 10).
Elmer W. Fisherman, M. D., Chicago, Ill.

29. **Tonsillectomy and Allergy** (Abstract No. 19).
Mary F. Lerner, M.D., and Harry Markow, M.D., Brooklyn, N. Y.

ADDRESS: "Grapes of Wrath"—The Problem of Nasal Polyps. Francis L. Lederer, M.D. (By Invitation). Professor and Head of Department of Otolaryngology, University of Illinois, College of Medicine, Chicago, Illinois.

30. **The Effect of Ataractic and Antihistaminic Drugs on the Experimental Skin Wheal** (Abstract No. 28).
John H. Toogood, M.D., London, Ontario.

31. **Experimental Depletion of Mononuclear Cells in Guinea Pigs** (Abstract No. 32).
Rudolf E. Wilhelm, M.D., J. Prochaska Fisher, M.D., and Robert A. Cooke, M.D., New York, N. Y.

32. **Leucocytolysis: I. Lysis In Vitro in Presence of Pollen and House Dust Antigens** (Abstract No. 22).
Horace Pettit, M.D., Howard E. Sullivan, M.D., and Eleanor D. Hart, B.S., Ardmore, Pa.

33. **Transfer of Pollen Hypersensitivity From Man to Mouse With Leukocytes and Plasma** (Abstract No. 26).
Margaret F. Oakley, B.S., and Clement J. Sullivan, M.D., St. Louis, Mo.

TITLES OF PAPERS FOR WHICH PRESENTATION TIME WAS NOT AVAILABLE

34. **Serologic Studies Following Prophylactic Tetanus Antitoxin.** Carl E. Arbesman, M.D., S. Zwi Kantor, M.D., Buffalo, N. Y.

35. **Comparative Studies of Various Corticosteroids in the Treatment of Allergic Disorders.** Carl E. Arbesman, M.D., and Robert J. Ehrenreich, M.D., Buffalo, N. Y.

36. **Homologous Lung and Bronchial Antibodies in the Guinea Pig.** I. Leonard Bernstein, M.D., and Ralph Kelley, B.S., Cincinnati, Ohio.

37. **Evaluation of Gamma Globulin as a Therapeutic Agent in Infectious Asthma.** Earl B. Brown, M.D., and Anne Botstein, M.D., Scarsdale, N. Y.

38. **Comparative Study of Hexadecadrol (Decadron) and Prednisolone. Double Blind Clinical Study Involving Ragweed Pollenosis.** Earl B. Brown, M.D., Charles Popovitz, M.D., and A. B. Seigelaub, B.A., Scarsdale, N. Y.

39. **Dexamethasone (Decadron), a New Anti-Inflammatory Steroid in the Management of Allergic Disease.** Sidney Friedlaender, M.D., and Alex S. Friedlaender, M.D., Detroit, Mich.

40. **Comparison of Effects on Pulmonary Function in Pulmonary Emphysema and Bronchial Asthma of Triamcinolone and Hexadecadron.** Henry T. Friedman, M.D., Beverly Hills, Calif.

41. **Allergic Rhinitis, a Common Cause of Recurrent Epistaxis in Children.** Leonard S. Girsh, M.D., Philadelphia, Pa.

42. **The Relationship of Allergy to Heart Disease.** Aaron N. Gorelik, M.D., New York, N. Y.

43. **Studies on the Mechanism of Desensitization.** Andres Goth, M.D., Dallas, Texas.

44. **Asthma, Cortisone and Hypercortisonism.** Edmund L. Keeney, M.D., La Jolla, Calif., and Milan L. Brandon, Lieutenant (MC) USN, San Diego, Calif.

45. **Drug Allergy—A report of 200 Cases.** Mason I. Lowance, M.D., Atlanta, Ga.

46. **The Use of Respiratory Vaccines in the Management of Bronchial Asthma.** Angelo L. Maietta, M.D., Boston, Mass.

47. **Insects as Injectant Allergens.** Frank Perlman, M.D., Portland, Ore.

48. **The Incidence of Allergic Diseases in the Daily Practice of Three Venezuelan General Practitioners.** Carlos Benaim-Pinto, M.D., Caracas, Venezuela.

49. **A Physician's View of Allergy.** C. T. Piper, M.D., Adelaide, Australia.

50. **The Hygiene of Housing.** Theron G. Randolph, M.D., Chicago, Ill.

51. **Treatment of Status Asthmaticus Complicated by Vasomotor Collapse.** Milton Saier, M.D., Sidney Hecker, M.D., and Marcus Krupp, M.D., Palo Alto, Calif.

52. **Vasomotor Rhinitis of Pregnancy—A New Treatment.** Maurice H. Schulman, M.D., Boston, Mass.

53. **Infectious Asthma. Analysis of One Hundred Cases.** Oscar Swineford, Jr., M.D., Earl R. Johnson, Jr., M.D., and Henry Cook, Lieutenant Colonel, MC, USA, Charlottesville, Va.

54. **Specific and Non-Specific Passive Desensitization.** Oscar Swineford, Jr., M.D., and Susan Quelch, B.S., Charlottesville, Va.

55. **Studies in Atopic Dermatitis. V. Problems With Inhalant Hyposensitization.** Louis Tuft, M.D., Philadelphia, Pa.

56. **Inhibition of Hystamin Shock in Guinea Pig by Citric Acid and Niacin Amide.** Vladislav S. Uzelatz, M.D., University of Belgrade, Yugoslavia.

57. **Studies of the Action of Methylprednisolone in Bronchial Asthma. A Comparison of Oral Methylprednisolone, Intravenous Methylprednisolone, Epinephrine, and Triamcinolone.** Thomas E. Van Metre, Jr., M.D., Lawrence J. Rosen, M.D., and Arnold F. Lavenstein, M. D., Baltimore, Md.

58. **Higher Co-Seasonal Dosage Levels in the Treatment of Pollenosis.** Bernard M. Zussman, M.D., Memphis, Tenn.

SCIENTIFIC EXHIBITS

Aerobiology—Technics and Applications. Pollen and Mold Committee of The American Academy of Allergy.

Insect Allergy—The Importance of Insects as a Cause of Respiratory Allergic Disease. Richard D. Wiseman, M.D., William G. Woodin, M.D., and Howard C. Miller, Ph.D., Syracuse, N. Y.

In Vitro Demonstration of Antibodies in Allergic Sera. Joan Givas, J. Gordon, Bram Rose, M.D., and A. H. Sehon, Ph.D., Montreal, Quebec.

Natural Food as Skin Testing Materials. Stanley S. Freedman, M.D., and Aidan J. Kavanagh, M.D., Providence, R. I.

Air Processing. Harry L. Huber, M.D., Chicago, Ill., and Willard S. Small, M.D., Pasadena, Calif.

Acute Obstructing Bronchiolitis—A Medical Emergency. E. H. Morgan, M.D., H. R. Pearsall, M.D., and H. L. Wilson, M.D., Seattle, Wash.

Medicine Advances With Safe New Drugs. Irvin Kerlan, M.D., John W. Nesbitt, M.D., and Albert H. Holland, Jr., M.D., Washington, D. C.

Cytology of Inflammatory Conditions of the Nose. William T. K. Bryan, M.D., and Marian P. Bryan, A.B., St. Louis, Mo.

The Adrenocorticotropic Hormone—Chemistry, Physiology, and Therapeutic Uses. R. C. Merrill and K. W. Thompson, M.D., Orange, N. J.

ABSTRACTS OF PAPERS

1. THE EFFECT OF HYPOSENSITIZATION THERAPY ON THE HEMAGGLUTINATING ANTIBODIES IN RAGWEED SENSITIVE PATIENTS

Carl E. Arbesman, M.D., S. Zwi Kantor, M.D., Robert B. Beede, M.D., and Noel R. Rose, M.D., Buffalo, N. Y.

Sera were obtained from seventy-eight ragweed-sensitive patients prior to specific hyposensitization therapy. Subsequent sera from each of these patients was taken just prior to and at the end of the ragweed pollen season. As controls, sera from nine untreated ragweed-sensitive patients were available before and after the pollen season. All of these sera were frozen until ready for study.

The hemagglutinating titers, as determined by both the tanned cell and bis-diazatized benzidine techniques, were measured for all samples of sera.

Many of the untreated sera had definite, demonstrable, reproducible hemagglutination titers, but usually the titers were markedly increased following specific hyposensitization therapy.

The effect of specific ragweed injections, the amount of extract administered, and the exposure to the inhaled pollen (during the season) on the hemagglutination titer will be determined and reported. Also, the relationship of the results of therapy to this hemagglutinating titer is being evaluated.

Comparative titers by each of the two methods, bis-diazotized benzidine, and tanned cell will be discussed as will the relative merits of each technique.

Further studies with each of these sera, to determine the reaginic titer and blocking antibody and their relationship to the hemagglutinating antibody, results of therapy, and dosages of treatment, are also in progress and we anticipate reporting on this at the same time.

2. PULMONARY FUNCTION IN NORMAL AND ASTHMATIC CHILDREN

I. Leonard Bernstein, M.D., Louis Kreindler, M.D., Joseph E. Ghory, M.D., Moshe Gerond, M.D., and Ronald G. Fragge, M.D., Cincinnati, Ohio

Soon after the inception of a long-term hospitalization program for chronically asthmatic children at the Cincinnati Convalescent Hospital for Children, it became apparent that the objective assay of pulmonary function in these children would serve as a valuable guide in the management and prognosis of these patients. Therefore a pulmonary function unit was established with facilities to measure lung volumes, ventilatory function, distribution of inspired air, and the mechanics of respiration. All measurements were performed in a Collins 9 liter closed-circuit apparatus with addition of a Katharometer and helium indicator for determination of the functional residual capacity.

Data on fifty normal children in the age range of 6 to 14 will be presented. These will include total vital capacity, timed vital capacity, maximum breathing capacity, inspiratory capacity, expiratory reserve volume, total lung capacity, functional residual capacity, residual volume, RV/TLC ratios, tidal volume, minute volume and alveolar ventilation.

In addition data on a smaller series of asthmatic children will be discussed. Physiologic measurements were done on many of these children soon after admission to the hospital and after long-term hospitalization.

3. ON MODIFICATION OF PENICILLIN ANTIGENICITY

George H. Berryman, M.D., and Elmer W. Fisherman, M.D., Chicago, Ill.

1. With a passive transfer technique it has been shown that serum reagins to penicillin G or V do not react with synnematin or penicillamine.

2. Direct intradermal tests in two penicillin G sensitive individuals were positive to penicillins G and V, but negative to synnematin and penicillamine.

3. It is concluded that modification of the chemical structure of the penicillin molecule brought about in replacement of the cyclic benzyl or thenyl group by a straight chain 4-amino 4 carboxy-n-butyl group results in loss of cross-antigenicity. Degradation of the penicillin molecule to produce penicillamine (di-methyl cysteic acid) also results in loss of cross-antigenicity.

4. The influence of chemical structure upon the immunologic and biologic potentialities of the penicillin molecule is discussed.

4. IMMUNE SERA IN THE STUDY OF FOOD ANTIGENS. I. FISH. II. CRUSTACEANS

SHELDON G. COHEN, M.D., WILKES-BARRE, PA.

The specificity of precipitinogenic activity of antisera from immunized rabbits was applied to the qualitative antigenic analyses of selected fish and edible shellfish extracts, fish-derivative pharmaceuticals, fish emanations and "insect-type" crustaceans utilizing agar-gel diffusion studies.

(1) *Fish-derivative pharmaceuticals:* Varying numbers of the five or six identifiable antigenic fish fractions were demonstrated in some lots of cod-liver oil, haliver oil, sodium morrhuate, and protamine.

(2) *Fish emanations:* Antigenic fish constituents could not be demonstrated in volatilized preparations derived from raw or cooking fish.

(3) *Fish roe:* Lipoproteins biochemically related to chicken egg yolk demonstrated species specificity.

(4) *Fish and shellfish skin test extracts:* There was a marked lack in the uniformity in number of individual antigenic components found in extracts prepared in different laboratories.

(5) *Edible crustaceans:* Species specificity and only partial cross-antigenicity were noted among the multiple component antigens of edible muscle portions of lobster, crab, shrimp, rock lobster, and fresh-water crawfish.

(6) *"Insect-type" crustaceans:* Varying degrees of partial qualitative antigenic relationship was noted for tiny brine shrimp and water fleas (daphnia) and taxonomically related edible shellfish of salt- and fresh-water habitats.

Possible interpretations of these findings suggest that important determinants of skin-test reactivity and allergic manifestations to shellfish or fish and their derivative preparations may include the variable presence of the particular component antigens for which the patient possesses specific reagins and/or clinical sensitivity along with a quantitative antigenic requirement.

5. THE SPATIAL VECTORCARDIOGRAM IN BRONCHIAL ASTHMA—A CLINICAL AND CARDIOGRAPHIC STUDY

LEO H. CRIEP, M.D., AND MARVIN SILVERBLATT, M.D., PITTSBURGH, PA.

The purpose of this study is to determine what changes, if any, occur in the spatial vectorcardiogram in both complicated and uncomplicated bronchial asthma. These changes are related to a complete medical, allergic, and cardiologic survey in this series of patients.

The vectorcardiogram reveals the three-dimensional orientation in space of the single electrical field surrounding the heart. It will occasionally supply information which is not made available by the electrocardiogram, i.e., (1) the presence of heart muscle changes, (2) rotation patterns of the electrical field characteristic of chronic pulmonary disease, (3) subtle evidence of right ventricular hypertrophy, and (4) evidence of right ventricular preponderance.

The study was carried out on 110 cases of bronchial asthma. Twenty-four patients presented pulmonary complications. In thirty-four patients the duration of the disease was over ten years. Whereas in a previous study by one of us (L. H. C.) uncomplicated bronchial asthma showed no electrocardiographic changes, we find in this series that about one-third of the patients displayed distinct vectorcardiographic evidence of, if not an abnormality, a definite

variant from the usually encountered pattern seen in most nonasthmatic normals. These changes may be the most subtle electrical evidence of right-sided pressure increases possibly representing syncytial muscle hypertrophy in the region of the crista supraventricularis.

In the group of patients showing pulmonary complications of long-standing bronchial asthma, three series of vectorcardiographic alterations were noted:

Patterns suggestive to represent right ventricular preponderance of an acquired type were encountered in a few cases. This finding was compared with the evidence obtained by one of us (M. S.) earlier on the production of right ventricular preponderance by congenital lesions (interatrial septal defects) a study corroborated by cardiac catheterization. Other vectorcardiographic evidence of various types of right ventricular preponderance was found in a small percentage of our patients. These have been correlated grossly with right-sided systolic pressure increases.

Other changes included, in a smaller number of patients, evidence of right atrial preponderance and the following: QRS frontal plane forces not only oriented in the +60 to plus/minus 180 degree segement but in the extreme right superior quadrant of plus/minus 180 to minus 90 degrees.

This study contributes to the available information as to cardiac changes observed in complicated and uncomplicated bronchial asthma.

6. FEVER AS A MANIFESTATION OF AN EXPERIMENTAL ALLERGY

Richard S. Farr, M.D., Pittsburgh, Pa.

Fever following the parenteral administration of various agents is sometimes a manifestation of human allergy, and the febrile response of sensitized rabbits to pyrogen-free bovine serum albumin (BSA) appears to provide an experimental model for the study of a similar allergy in animals. This system is unique since many of the known variables involved can be quantitated. The febrile response can be quantitated by means of a fever index; the crystalline BSA is a moderately well-defined antigen, the fate of which can be followed at physiologic concentrations by means of an I^{131} isotope label; and the quality as well as quantity of circulating antibody can also be studied. Bacterial endotoxins probably do not play a role in the BSA fevers, since no cross-tolerance could be demonstrated between the BSA and endotoxin febrile responses.

The febrile response to BSA is thought to represent a true altered response, or rabbit allergy, and not an intoxication, an idiosyncrasy, or a regularly occurring physiologic response to in vivo antigen-antibody reaction because

(a) Normal rabbits did not develop fever after the first intravenous injection of 1 to 10 mg. BSA.

(b) Within a group of uniformly sensitized rabbits, approximately one-half did not develop significant fever when challenged with BSA a month after the last sensitizing dose.

(c) Whether a given rabbit responded to fever seemed less dependent on the method of immunization than on the individual animal itself.

The antibody content of the sera of these animals was studied with the quantitative precipitin test as well as the ammonium sulfate method of measuring antigen-binding capacity. All of the rabbits responding with fever, and many not responding with fever, had circulating antibody. Rabbits without

circulating antibody did not develop fever; otherwise, there was no correlation between *total* antibody per milliliter of serum and febrile responsiveness. However, by means of the ammonium sulfate method, it was possible to show that the rabbits with considerable amounts of circulating antibody, but with minimal febrile responses, produced antibody which was predominantly of a quality which would bind antigen efficiently in dilute solution. Conversely, rabbits that produced antibody which could not bind antigen efficiently in dilute solution were those with the largest febrile responses. Whether circulating antibody was acting by binding the challenge dose of BSA before it could reach a sensitized cell population or whether a particular quality of circulating antibody was directly involved in the actual production of fever remains to be determined.

7. AN IMMUNO-CHEMICAL METHOD FOR THE DETERMINATION OF THE SERUM LEVEL OF INSULIN

Richard J. Feinberg, M.D., Chicago, Ill.

Biologic methods for the detection of small quantities of insulin are dependent upon the biologic variation of the test animal or tissue used in the assay. However, the most important objection to these methods is that they measure the resultant of insulin and its antagonists, rather than a true level of insulin. The Boyden technique of adsorbing protein onto tanned erythrocytes was applied to an insulin system and found to be feasible. The antiserum used should be specific; therefore, purified, glucagon-free, physiologically active insulin was used as antigen, to prepare the antiserum, and as insulin, adsorbed onto the tanned erythrocytes. Excess soluble insulin was shown to inhibit the agglutination of insulin-coated RBC by the anti-insulin serum. The physiologic activity of insulin, that of shocking mice, was neutralized by the addition of the anti-insulin serum to the system. A final test for the anti-insulin serum was the inhibition of the action of insulin in increasing the uptake of glucose by the isolated rat diaphragm. The specific inhibitory effect of free soluble protein on agglutination of tanned, antigen-treated erythrocytes by antisera was noted by several workers. This paper outlines the technique used for detecting levels of insulin as low as 0.003 micrograms (75 to 80 microunits). The question of specificity will be discussed, as will be the importance of separating physiologically active from physiologically inactive insulin. Use of the technique for detecting serum insulin levels in normal human beings and cats, diabetic human beings on tolbutamide, normal human beings after a glucose load, and hypophysectomized and depancreatized cats will be reported. The applications of the system to insulin-resistant individuals will be discussed.

8. INHIBITION OF ANAPHYLAXIS IN THE GUINEA PIG BY LOCAL STREPTOCOCCAL INFECTION. THE INFLUENCE OF ADRENALECTOMY

Leo N. Meleyco, M.D., Jacob J. Pruzansky, Ph.D., Samuel M. Feinberg, M.D., and Samuel Z. Kantor, M.D., Chicago, Ill.

In studies on anaphylaxis in the guinea pig it was occasionally observed that control animals were protected against anaphylaxis. A correlation was noted between reduction in anaphylactic reactivity and the presence of a naturally occurring local abscess from which Group C streptococci could be

isolated. Under carefully controlled conditions this inhibitory phenomenon could be reproduced in large numbers of uninfected animals inoculated with a pure culture of this streptococcus.

In man partial or complete remission from allergic symptoms has been observed during and following acute infections or other conditions which have been classified as stress. Some of the manifestations of stress are currently considered to be produced through pituitary-adrenal stimulation. Furthermore, ACTH and cortisone or other similar steroids have proved effective in the management of various allergic conditions, suggesting that increased steroid hormone production is the mechanism for the clinically observed reduction in hypersensitivity during infection. For this reason, a study was undertaken to determine what effect bilateral adrenalectomy would have on the observed protection of the guinea pig against anaphylaxis following local infection with a hemolytic streptococcus.

The results show that guinea pigs in which a local streptococcal abscess is induced are protected from anaphylaxis whether the adrenals are intact or are completely removed. The inhibition cannot be ascribed to a greater tolerance to histamine or to nonspecific effects of the operative procedure itself. The possible implications of these findings will be discussed.

9. STUDIES OF THE ANTIGENIC RELATIONSHIP OF INSECT EXTRACTS BY REAGIN NEUTRALIZATION. EVIDENCE FOR NONSPECIFIC INACTIVATION OF REAGIN

JACOB J. PRUZANSKY, PH.D., SAMUEL M. FEINBERG, M.D., ARTHUR KAMINKER, M.D., AND LEO N. MELEYCO, M.D., CHICAGO, ILL.

As part of our study of the immunologic relationship of extracts of various insects, we included the method of neutralization of passively transferred reagin in nonatopic subjects. Each extract was titrated on a series of sensitized sites. The concentration used for desensitization and testing was the one which gave the first ++ or +++ reaction. All sites were desensitized with a specific extract until a test dosage no longer reacted on the site. For 1 to 2 weeks the sites were not reactive to Compound 48/80 in doses reacting ++ in virgin skin. When reaction to 48/80 returned (thus showing evidence of return of available histamine or other mediators), it was found by extensive cross-testing of insect extracts that complete cross-desensitization had taken place. This suggested the possibility of extensive immunologic cross-relationship. Reactions to ragweed and alternaria (against which reagins were also present), however, were also negative, proving that nonspecific rather than specific desensitization had occurred.

Reactivation of sites could be obtained by reinjection with the reaginic serum. This indicated that in some way all reagins in the originally transferred serum had been nonspecifically inactivated during the "specific" desensitization procedure. Production of nonspecific skin reactions by histamine or by 48/80 in sensitized sites parallel in size and frequency with sites that were specifically desensitized also resulted in inactivity to all allergenic extracts. These sites could also be reactived by introducing reaginic serum. This reactivation by reagin again suggests that the skin of the subject was completely adequate for reaction and the only factor missing was the reagin. It would appear, therefore, that nonspecific desensitization has been shown to be due to depletion of reagin. These findings suggest at least two practical implications: (1) the hazards in interpretation of passive transfer studies for purposes of evaluating antigenic relationships and (2) the possible therapeutic significance of heterologous and nonspecific "desensitization."

10. DOES THE ALLERGIC DIATHESIS INFLUENCE MALIGNANCY?

ELMER W. FISHERMAN, M.D., CHICAGO, ILL.

Various studies suggest that natural defenses against cancer exist. These may explain the differences between cancer-prone and cancer-free individuals and differences in rate of tumor growth, invasiveness, cureability, and on very rare occasions spontaneous disappearance.

Recently, immunologic factors have been implicated in carcinogenesis as well as in transferability of tumors after development. Green has proposed that the essential prerequisite for malignancy is an initial loss of "identity antigens" which he believes are identical with the "self-markers" of Burnets and Fenners' theory.

It has also been suggested that tumor tissue, if sufficiently differentiated from the host's normal tissue, might induce the formation of cytotoxic auto-antibodies.

Though it is likely that the relationship between host and invading tumor is complex and cannot be defined in simple terms, it became of interest to survey the incidence and progress of the malignant process in groups of a population which are distinguished by a single immunologic characteristic, atopy, which may or may not be involved in the basic defense mechanism of the body.

Rigid criteria for atopy were established. Patients with cancer were interviewed at random and compared with control noncancer patients of the same age group. Malignant tumors were classified according to increasing severity: 1 plus to 4 plus. Actual survival was compared with expected survival.

Results on 800 cancer patients to date reveal an incidence of approximately 3.25 per cent as compared with 12.5 per cent in noncancerous patients. The incidence of active atopic disease in the general population has been estimated at 10 per cent.

The study indicates that atopic disease occurs in cancer patients but that the incidence is significantly lower than expected. Moreover, close to 40 per cent of the patients had noted disappearance of atopic symptoms approximately ten years prior to the onset of the malignancy. Few had severe allergies. Patients with cancer of the uterus, breast, and prostate showed a particularly impressive decrease in the incidence of atopy.

11. CUTANEOUS REACTIONS TO AUTOLOGOUS AND NONAUTOLOGOUS PLASMA

SAMUEL O. FREEDMAN, M.D., J. Q. BLISS, M.D., J. L. HUTCHISON, M.D., AND A. S. V. BURGEN, M.D., MONTREAL, QUEBEC

It is well known that iso-antigens associated with the formed elements of the blood are of importance in the causation of transfusion reactions. It is, however, generally assumed that human plasma is nonantigenic for human recipients.

In order to investigate the validity of this assumption, fifty human subjects who had never received transfusions of whole blood or plasma were skin tested with their own plasma (autologous plasma) and with plasma from several normal human blood donors (nonautologous plasma). Before skin testing, 50 mg. of Evans blue dye (T 1824) was administered intravenously.

It was found that extravasation of the dye into the surrounding skin occurred within thirty minutes at the nonautologous injection sites, but there

was no extravasation at the autologous sites. Similar experiments performed in dogs have shown that the same distinction between autologous and nonautologous plasma exists in this species and that the localized increased capillary permeability is probably due to histamine release.

One atopic human subject who showed a strongly positive cutaneous reaction to a particular nonautologous plasma was given a transfusion of 500 ml. of the same plasma and immediately developed giant urticaria. He suffered no ill effects from a transfusion of 500 ml. of his own plasma.

The exact significance of these findings is not clear at the present time, but they suggest that individual human plasmas may contain distinctive antigens similar to those found in tissue transplantation studies and that these antigens may account for some of the allergic reactions occurring in otherwise compatible blood transfusions.

12. FOOD ALLERGY: A STUDY OF 150 "ALLERGIC" CHILDREN

STANLEY S. FREEDMAN, M.D., AND WILLIAM SELLARS, M.D., PROVIDENCE, R. I.

This study began in early 1955 and ended in early 1958. One hundred and fifty consecutive allergic children were selected for study. All but four or five had a well-established allergic disease, the average duration of which was two years and nine months at the time of their initial visit. There were ninety-seven children with atopic eczema and/o. asthma. These children were picked for detailed study. There were numerous sensitivities encountered in this group besides food, such as pollen, mold, etc., but the object of this study was to determine the incidence of proved food sensitivity and the role it plays in the diseases from which these children suffered. Of the ninety-seven children with atopic eczema and/or asthma, sixty-seven produced preliminary evidence of food sensitivity. This initial evidence was based on positive skin tests to food, a positive clinical history of food sensitivity, or both. Of the sixty-seven children who gave preliminary evidence of food sensitivities, forty-seven were proved to be sensitive to 121 foods. Final proof of the existence of clinical food sensitivity was based on either a convincing positive history or the production of typical allergic symptoms by test feedings on at least three occasions at intervals of several weeks done when the child was comparatively asymptomatic.

The manifestations of food sensitivity were usually not related to the eczema or asthma from which these children suffered. In only one instance was a food (milk) apparently the sole cause of a case of asthma.

Skin tests were performed by the scratch technique. In addition to commercial food extracts, natural foods were also used as skin-testing materials. It was found that natural foods often gave stronger test reactions than commercial extracts. At times natural foods gave positive reactions when commercial extracts produced negative reactions. It is believed, therefore, that natural foods should be given further trials as skin-testing materials.

13. RATE OF RECOVERY OF NORMAL ADRENAL CORTICAL FUNCTION AFTER DEXAMETHASONE THERAPY

HENRY T. FRIEDMAN, M.D., AND JOHN F. MURRAY, M.D., BEVERLY HILLS, CALIF.

A series of patients requiring short-term steroid therapy was chosen. Initial blood samples were taken; then a four-hour maximal adrenal stimulation test was performed by giving 25 units of ACTH in 500 ml. of dextrose in water intravenously. A second blood sample was taken at this time. The patients were given 0.5 mg. hexadexadron, four times per day for one week.

A blood sample was taken at the identical hour of the initial sample and every four days thereafter. If, two weeks after the cessation of therapy, blood levels of 17-hydroxycorticosteroids did not reach pretreatment levels a second ACTH stimulation test, identical to the first one, was carried out.

A modification of the Nelson-Samuels technique for the measurement of serum 17-hydroxycorticosteroids was utilized. All bloods were fractionated into plasma and frozen immediately after withdrawal.

Potent synthetic adrenal cortical-like steroids which markedly depress physiologic function of the adrenal glands by suppressing endogenous ACTH production are widely used today. The controversy over how to stop dosage and whether or not ACTH should be used is unending. This is an experiment to measure quantitatively in man the actual functional depression produced and the time required for either the glands to spontaneously reacquire normal function and/or response to maximal ACTH stimulation. Thus in short-term steroid therapy physicians will know how to treat or to await normal functional returns of the adrenals.

14. THE RELATIONSHIP BETWEEN BRONCHIOLITIS AND CHILDHOOD ASTHMA

Heinz J. Wittig, M.D., Cranford, N. J., and Jerome Glaser, M.D., Rochester, N. Y.

Bronchiolitis, also commonly termed "capillary bronchitis," is an important disease of infancy. It is commonly due to some type of acute febrile infection accompanied by emphysema and wheezing respirations suggestive of asthmatic bronchitis. Multiple attacks rarely occur. Since the relationship of this disease to asthma in later childhood had never been investigated, the authors attempted a follow-up study of 181 patients with this condition seen in our hospitals between 1950 and 1957. One hundred patients were successfully traced. Asthma subsequently occurred in 32 per cent of these children and other allergic respiratory diseases occurred in an additional 17 per cent of children who did not develop bronchial asthma, resulting in a total incidence of 49 per cent of allergic respiratory disease. Of eighty-three families of these children, where the family histories could be determined with accuracy, allergic disease was present in 44 per cent, as compared with an incidence of allergic disease in normal families of 10 per cent. It is suggested that bronchiolitis in infancy should be looked upon as a condition which is often followed by the development of allergic respiratory disease, particularly asthma, and appropriate prophylactic measures should be taken.

15. SEDIMENTATION STUDIES OF SKIN-SENSITIZING ANTIBODY

Ernest M. Heimlich, M.D., W. E. Vannier, M.D., Ph.D., and Dan H. Campbell, Ph.D., Pasadena, Calif.

There has been some doubt as to whether skin-sensitizing antibody is associated with S^{19} heavy molecular weight material or with material of the S^{6-7} fraction of human serum. Sedimentation studies were performed on sera of twelve allergic patients variously sensitive to pollens, epidermals, house dust, and other inhalants.

These sera were separated in the preparative centrifuge into a pellet and a supernatant fraction by centrifugation at 40,000 r.p.m. for ten to twelve hours. The pellet material was passed through four further cycles of resuspension in borate-saline and recentrifugation as above. Supernatant material

was likewise recentrifuged four times, any additionally sedimented pellet material being discarded at each centrifugation. Examined in the analytic centrifuge, the final pellet fraction showed a large S^{19} peak and a small S^{6-7} peak. The final supernatants showed only S^{6-7} peaks.

Employing the Waugh cell in the analytic centrifuge, sera were divided into a "top" S^{19} free and a "bottom" S^{19} containing fraction. The top fraction was divided a second time in the Waugh cell to assure purity.

All fractions prepared by the two above methods were tested by passive transfer skin testing. Two separate nonsensitive recipients were used for each serum and fractions thereof.

Skin-sensitizing activity was found to be associated with all fractions tested including those devoid of the S^{19} component.

16. THE EFFECT OF HEAT PROCESSING AND GAMMA RADIATION ON THE ANAPHYLACTOGENIC PROPERTIES OF MILK

H. F. Kraybill, Ph.D., Denver, Colo.

Various methods have been tried in an attempt to induce molecular alteration of certain food proteins and ultimately effect a decrease in the allergenicity as well as an improvement in the digestibility of milk proteins. The effectiveness in the biochemical alteration of milk protein or other proteins can be measured by several precise chemical procedures as well as being quantitated through biologic testing with guinea pigs sensitized to the specific antigen under investigation.

Much attention has been focused upon the application of gamma radiation in the molecular transformation with the subsequent alteration in antigenicity. Skim milk has been gamma irradiated at radiation doses of 0.465, 2.79, 5.58 and 9.30 megarads, using mixed fission products as the radiation source. A reduction in the anaphylactogenic properties of skim milk proteins occurs with an increase in the radiation dose. A comparison of the ratios of the mean lethal shocking dose of LD^{50} values, expressed in micrograms of protein nitrogen, for unirradiated and irradiated milk samples shows biologically only a twofold change at 2.79 megarads, a ninefold change at 5.58 megarads, and a highly significant alteration of thirty-one fold at the 9.30 megarad dose level.

Fluid milk similarly processed with high-speed electrons to a dose level of 9.30 megarads utilizing a concurrent radiation distillation technique had acceptable properties as to odor, flavor, and colloidal stability. This product, however, on the basis of biologic testing (gross anaphylaxis), had an LD^{50} value of 130.8 micrograms of protein nitrogen, which is a fivefold change over that of the LD^{50} value for unirradiated or control skim milk.

Heat processing of milk for at least thirty minutes above 100° C. seems quite effective in the molecular alteration and subsequent reduction in the biologic response of milk-sensitized animals.

Further evidence on the magnitude of the molecular alteration of milk protein by these processing methods has been obtained by electrophoretic patterns and physical changes, such as viscosity, obtained on the milk protein samples. Under the influence of gamma radiation, milk protein undergoes denaturation, fragmentation, and eventually a coalescence of smaller fragments to large polymers, characterized electrophoretically. With this transformation, a destruction of the original antigenic factor occurs.

17. ABNORMAL SWEAT ELECTROLYTES IN PATIENTS WITH ALLERGIES

GILBERT LANOFF, M.D., OLIVER CRAWFORD, M.D., MARVIN SMOLLER, B.S., AND DAVID YI-YUNG HSIA, M.D., CHICAGO, ILL.

In the course of some studies on the frequency of the genes for cystic fibrosis of the pancreas, an abnormality has been encountered among patients with various forms of allergies.

All of the patients with allergies at The Children's Memorial Hospital were tested for Palmar sweat chlorides by the plate method described by Shwachman and Gahm. It was found that forty-two out of eighty patients (52.5 per cent) had readings of 3+ or 4+ (representing a chloride concentration of 75 mEq./1. or higher). In contrast, only 109 out of 986 control subjects (11 per cent) had readings of 3+ or 4+. The difference is highly significant (X^2 [1d.f.] = 56; p less than 0.001).

Many of the patients were then subjected to tests for sweat sodium and chloride performed in the usual manner. These were compared with those done on normal controls and children with cystic fibrosis of the pancreas. The results, which are expressed as mEq./1., were as follows:

	SODIUM		CHLORIDE	
	NO.	MEAN SE	NO.	MEAN SE
Controls	83	21.7 ± 1.9	83	19.5 ± 1.3
Allergic patients	31	42.8 ± 5.1	25	30.0 ± 3.7
Cystic fibrosis	29	103.2 ± 3.4	29	101.0 ± 4.3

It was found that eight out of the thirty-one allergic patients had sweat sodium levels of 50 mEq./1. or higher, while only six out of the eighty-three normal controls exceeded that level (X^2 [1d.f.] = 5.0; p between 0.02 and 0.01).

The sweat abnormalities were not limited to patients with any one particular form of allergy. Also, they did not relate to the type of medication used, since many of the patients were on no therapy at all.

The allergic patients with abnormal sweat electrolytes were placed on cortisone therapy, and the sweat electrolyte determinations were repeated. Several of these patients were also intubated for duodenal enzymes. The results of these studies will be discussed.

18. SKIN-SENSITIZING ACTIVITY OF HUMAN SERUMS AND SERUM FRACTIONS

FREDERICK ALADJEM, PH.D., WALTER R. MACLAREN, M.D., ROBERT R. JAROSS, B.S., AND PADMISINI K. AYENGAR, PH.D., LOS ANGELES, CALIF.

Human serums and serum fractions were observed to produce, upon intradermal injection in man, wheal and erythema reactions indistinguishable from those produced upon challenge in passive transfer reactions. Volumes of 0.05 ml. were tested intradermally in volunteers. Wheal and erythema reactions reached maximum intensity within ten to twenty minutes.

It was found that serums or serum dilutions of most (though not all) individuals caused wheal and flare reactions. By starch electrophoresis, however, skin-reactive material could be isolated from all serums; this material had mobility intermediate between beta and gamma globulins. Its properties were similar to the active material isolated from rabbit serum (Aladjem,

MacLaren, and Campbell, *Science* 125: 692, 1957). It is not dialyzable, can be precipitated with 40 per cent ammonium sulfate, and is largely associated with water-insoluble globulins.

Autologous serums and serum fractions were found to be as reactive as homologous serums and serum fractions. This rules out immunologic incompatibility as a mechanism of the reaction. Antihistamines, crystalline trypsin inhibitor, and various human serum fractions, including the ones that contain the trypsin inhibitor, were not effective in reducing the wheal and flare reactions. Guinea pig passive cutaneous reactions and gut contraction can be used to assay the serum reactivities, but these reactions are much less sensitive than skin reactivity in man. Evidence will be presented that there appear to exist in human serums at least two systems that can be activated to cause wheal and flare reactions in man.

19. TONSILLECTOMY AND ALLERGY

MARY F. LERNER, M.D., AND HARRY MARKOW, M.D., BROOKLYN, N. Y.

The charts of seventy patients who are attending the Allergy Clinic of the Beth-El Hospital, Brooklyn, were reviewed. These patients had tonsillectomies before joining the clinic and were considered in this study because sufficient information was obtained concerning the preoperative and postoperative course. Fifty were allergic and twenty were clinically nonallergic prior to the operation. The symptoms for which the tonsillectomies were done could be classified as allergic in the allergy patients and infective in the nonallergic patients. Following the operations, the fifty allergic patients remained in the same status without any improvement, except for two who were under allergic management and improved. The twenty clinically nonallergic patients developed allergic manifestations within one to twelve years following the operation. Within one year there appeared five cases of asthma, four cases of hay fever, two cases of rhinitis, and two cases of bronchitis, and within twelve years all had developed allergic manifestations. Of the fifty preoperatively allergic patients two improved following tonsillectomy but these were under preoperative allergic management; seven improved, but symptoms recurred in from two to four years; sixteen were unimproved; eight were worse; and seventeen patients developed new allergic manifestations—50 per cent in one year and 90 per cent in three years. Of greatest interest was the postoperative development of bronchial asthma in eight allergic and eight clinically nonallergic patients while the thirteen preoperative asthmatics remained asthmatic postoperatively.

It is our conclusion that asthma and other allergies are in themselves no indication for tonsillectomy, that tonsillectomy not only will not cure allergic diseases but may induce new allergic manifestations in allergic patients, that asthma not only is not cured by tonsillectomy but may be induced since sixteen nonasthmatic patients developed asthma after the operation, and finally that patients awaiting tonsillectomy should be thoroughly studied from every angle, including repeated cytologic studies of their respiratory secretions, to be sure that the operation is not done to relieve allergic symptoms instead of to remove infective tissue which acts as a focus of infection or as a trigger mechanism.

20. STUDIES ON THE SIMULTANEOUS INHIBITION OF ANAPHYLATOXIN FORMATION AND COMPLEMENT FIXATION

ABRAHAM G. OSLER, PH.D., BETSY M. HILL, SC.M., HARRY G. RANDALL, B.A., ZOLTAN OVARY, M.D., AND EUGENE RODRIGUEZ, B.S., BALTIMORE, MD.

The interaction of antigen with antibody in the presence of fresh rat or guinea pig serum may lead to a loss in complement (C) activity of the serum

with concomitant appearance of a substance called anaphylatoxin. This product of an in vitro C fixation reaction apparently mediates two manifestations of the tissue response characterizing allergic reactions of immediate type. These are enhanced capillary permeability as studied in the guinea pig skin and contraction of smooth muscle as seen in the Schultz-Dale bath.

It has been demonstrated that certain aspects of anaphylatoxin formation are entirely similar to the reaction steps described for the lysis of red blood cells by antibody and C. For example, inhibitors of C activity, such as phlorizin or chelating agents which deplete the reaction medium of essential cations, also suppress the formation of anaphylatoxin. Studies with these and other inhibitors provided evidence for the participation of the third component of C (C 3) in the production of anaphylatoxin. One of the implications which have emerged from experiments dealing with the role of C in allergic reactions of the immediate type points to the possibility of interposing a bloc between the in vivo antigen-antibody reaction and the tissue-damaging consequences of this event.

21. ANAPHYLACTIC REACTIONS IN THE SKIN OF THE GUINEA PIG WITH HORSE, SHEEP, AND CHICKEN ANTIBODIES

Zoltan Ovary, M.D., Baltimore, Md.

It has been shown that passive cutaneous anaphylaxis (PCA) can be elicited in the guinea pig with homologous, rabbit, and human antisera. In contrast, antibodies in horse, sheep, and chicken sera fail to provoke cutaneous or systemic anaphylactic reactions in the guinea pig. However, PCA takes place only when the antibody is injected at a sufficient time before the injection of the antigen. This latent period has been interpreted as the interval required for "fixation" of the antibody. When the antigen is injected before the antibody (reverse PCA), a variety of immune systems fail to evoke this reaction. However, Bier and Siqueira succeeded in producing reverse PCA in guinea pigs with homologous anti-rabbit gamma globulin sera. These findings were interpreted to indicate that the rabbit gamma globulin can be "fixed" to the cells of the guinea pig, not only when it is used as antibody but also when it is used as antigen. It was predicted that human gamma globulin could act similarly, since this species of antibody can provoke PCA in the guinea pig. It was therefore hoped that antisera against these gamma globulins could give reverse PCA reactions, regardless of the source of the antibody.

In the present study evidence is presented that reverse PCA reactions can be provoked with horse, sheep, and chicken antisera against either rabbit or human gamma globulins. These results indicate that anaphylactic reactions can be provoked in the guinea pig skin if either the antibody or the antigen is "fixed" to the cells. Although horse, sheep, and chicken antibodies are not fixed to the skin, they do react and produce PCA in the presence of rabbit or human gamma globulin when the latter two proteins act as antigen and have been fixed to the appropriate but still unknown cell site.

22. LEUCOCYTOLYSIS. I. LYSIS IN VITRO IN PRESENCE OF POLLEN AND HOUSE DUST ANTIGENS

Horace Pettit, M.D., Howard E. Sullivan, M.D., and Eleanor D. Hart, B.S., Ardmore, Pa.

Leucocytolysis in vitro in the presence of homologous serum and specific antigen remains controversial. To obtain further data in this field, heparinized blood from allergic patients and normal controls were exposed to pollen and

house dust antigens. Leucocyte counts were made immediately and after eighty minutes. The method used was based on that of Squire and Lee, with quantitative variation (Squire and Lee, J. ALLERGY 18: 156-162, 1947).

Standard deviation was applied to the results and the mean taken to compare the leucocyte counts of the specific blood-antigen systems to the controls. The mean of 227 specific blood-antigen systems showed that the leucocyte count in eighty minutes was 88 per cent of the count at 0 minutes. The mean of the matching controls showed that the leucocyte count in eighty minutes was 100 per cent of the count at 0 minutes.

The results obtained in this series of experiments apparently indicate that homologous leucocytes exposed to specific antigens in the presence of specific plasma for eighty minutes show a mean drop in count to 88 per cent of the 0 minute count. This drop seems to be sufficiently significant, when standard deviation and mathematical mean are applied to the system and the controls, to warrant further investigation.

23. THE ANTIGENICITY OF ESTRONE-PROTEIN CONJUGATES

BRAM ROSE, M.D., L. GOODFRIEND, AND A. H. SEHON, PH.D., MONTREAL, QUEBEC

Estrone has been coupled via stable covalent bonds to various proteins. The sera of rabbits immunized with these conjugates were shown to contain antibodies against the estrone residue by precipitin hemagglutination, PCA, and Dale-Schultz techniques. The details of the chemical and immunologic procedures, as well as the implications of these results, will be discussed.

24. METABOLIC STUDIES ON ASTHMATIC PATIENTS TREATED WITH NEWER STEROIDS

ELEANOR MCGARRY, M.D., ELEANOR VENNING, M.D., AND BRAM ROSE, M.D., MONTREAL, QUEBEC

Studies in a hospital on a metabolic ward were done on two patients with asthma prior to, during, and following treatment with newer steroids. Nitrogen, calcium, phosphorus, sodium, and potassium balances were done continuously. ACTH tests with measurement of blood and urinary corticoids were done before and after each course of treatment. Urinary 17-ketosteroids and 17-hydroxycorticoids were followed throughout the study. Both patients showed a good response to treatment as judged by clinical assessment and respiratory function studies. One patient, not previously treated with steroids, showed enhancement of a positive nitrogen and calcium balance while receiving 9α-fluoro-16α-hydroxy prednisolone (Triamicinopone). There was no change in adrenal responsiveness to ACTH, although there was depression of the 17-OH corticoids during treatment. The second patient, who also had severe atopic dermatitis treated with steroids for seven years, was followed over a period of 102 days. Both 9α-fluoro-16α-OH prednisolone and 6-methyl prednisolone in relatively large doses produced negative nitrogen and calcium balances which became positive when the dose was tapered to maintenance levels. Endocrine assays showed adrenal suppression prior to the experimental period with sluggish response to ACTH. There was no change in adrenal responsiveness after treatment with either of the drugs used. In neither patient was there sodium retention or potassium excretion during treatment. Further studies are in progress.

25. PARALLEL VOLUMETRIC AND GRAVIMETRIC POLLEN COUNTS. I. THE VARIABILITIES AND INCONSISTENCIES OF A CONVERSION FACTOR

GEORGE I. BLUMSTEIN, M.D., AND JAY SPIEGELMAN, M.D., PHILADELPHIA, PA.

Most pollen counts are now done by the gravity-slide method and converted to a volumetric equivalent by multiplying by a conversion factor.

During the 1957 ragweed season, two volumetric and one standard gravity air sampling devices were exposed side by side. The only variable was that one volumetric impinger drew 5 liters of air per minute while the other drew 10. Each apparatus gave forty-six daily calculations. These figures form the basis of this report, although the 1958 figures should also be available for comparison.

The daily pollen counts in the two volumetric apparatuses had a high degree of correlation. All volumes were reduced to their cubic yard values and the gravity slide counts to square centimeter values. A comparison was then made between the volumetric and gravimetric figures for each day.

The resultant figures showed a high degree of variability, ranging on one volumetric impinger from 0.7 to 25.0 with a mean of 8.8 and from 0 to 27.0 with a mean of 8.5 on the second impinger. When subjected to statistical analysis, a coefficient of variation of 55 per cent, too high to have statistical validity, was obtained for both factors. These factors are at variance with Durham's figure of 3.6 for ragweed pollen, but when his original figures were subjected to the same type of analysis the same high coefficient of variation of 55 per cent was obtained.

It is suggested that all future gravimetric counts be expressed in square centimeters of slide area and that conversion to a volumetric equivalent, which these studies show to be inconsistent, be avoided. It is further recommended that, where true volumetric figures are desirable, a satisfactory volumetric device be utilized.

26. TRANSFER OF POLLEN HYPERSENSITIVITY FROM MAN TO MOUSE WITH LEUKOCYTES AND PLASMA

MARGARET F. OAKLEY, B.S., AND CLEMENT J. SULLIVAN, M.D., ST. LOUIS, MO.

Convulsions, and rarely death, after challenge with the specific pollen antigen occurred in a small percentage of pertussis-inoculated mice prepared with leukocytes from human donors with pollen allergy. Control mice prepared with the donor's plasma prior to pollen antigen challenge developed convulsions slightly less frequently. With leukocyte preparation, the incidence was 1/12, 0/12, 0/12, 0/13, 5/13, 1/13, and 2/6. With plasma preparation, the incidence was 0/6, 0/6, 0/6, 0/4, 1/4, 2/6, and 1/3. Separation of plasma from leukocytes was effected as completely as possible by centrifugation and decanting the plasma; in only the first of the seven challenges had the leukocytes been washed. Failure to obtain high yield of viable leukocytes in earlier experiments led to the abandoning of washing the leukocytes. Intravenous reinjection of the antigen twenty-four or six hours after an initial intraperitoneal or intravenous challenge was followed by relatively high incidence, 5/13 and 2/6, of convulsions. High degree of clinical hypersensitivity of the donor and the absence of any previous specific therapy in this donor were thought to influence the high frequency of occurrence of convulsions in mice prepared with his leukocytes, 5/13; with his plasma, the occurrence was 1/4.

The ability to transfer pollen anaphylactic hypersensitivity from human donors to mice is probably demonstrated. The evidence suggests that leukocytes as well as plasma are capable of tranferring the hypersensitivity. The possibility that traces of plasma may have effected the transfer in all cases has not been disproved by the method used.

27. PURIFICATION OF ANTIBODIES: STUDIES OF ANTIBODIES ELUTED FROM DEXTRAN-TYPE II PNEUMOCOCCAL ANTISERUM PRECIPITATES BY TREATMENT WITH DEXTRANASE

Oscar Swineford, Jr., M.D., Rudolf Hoene, M.D., Ph.D., and Susan Quelch, B.S., Charlottesville, Va.

Purification of antibodies is a persistent challenge. Purified antibodies are needed for current studies of the antibody which provides specific passive desensitization against anaphylactic shock. Preliminary tests showed that dextranase destroyed the dextran in dextran Type II antipneumococcal precipitates. This eluted antibody was subjected to a series of reprecipitations and repurifications with the following results:

Dextranase denatured dextran in simple solution, in solution with sensitizing antibody, and in insoluble complexes with precipitated antibody.

Eighty-six per cent of 100 gamma of antibody nitrogen was reprecipitated and repurified.

Dextran, in the zone of antigen excess, precipitated only a part of the antidextran sensitizing and precipitating antibodies from "purified" antibody solutions.

Purified antibody precipitated regularly with Type II polysaccharide but did not sensitize against it.

Purified antibody precipitated with and sensitized against dextran after two dextranase treatments. After the third purification, the antibody still precipitated strongly with dextran but did not sensitize against it. The purified antibody, in large amounts, failed to provide passive desensitization against dextran.

Evidence is offered that the polysaccharide is the dominant hapten in the Type II polysaccharide-dextran-specific antiserum system. The idea of dominant haptens is a new one.

Purification of antibodies by enzymes should be pursued in other immune systems.

28. THE EFFECT OF ATARACTIC AND ANTIHISTAMINIC DRUGS ON THE EXPERIMENTAL SKIN WHEAL

John H. Toogood, M.D., London, Ontario

Antiwhealing properties of chlorpromazine (Thorazine) and chorprophen-pyridamine maleate (Chlor-Trimeton), were previously demonstrated by us, using a graded-response bioassay technique. This technique lends itself well to quantitative comparison between drugs. These observations have been confirmed and extended by further experiment in the dog and in man and compared with the effects of perphenazine (Trilafon) and hydroxyzine (Atarax). Some degree of antiwhealing effect is demonstrable with all drugs tested, being conspicuously greatest in the case of Atarax. The latter finding agrees with recently published reports by Feinberg and colleagues of the clinical superiority of hydroxyzine over some other tranquillizers and antihistamines in certain cases of chronic urticaria.

29. EFFECT OF SPECIFIC DESENSITIZATION ON IN VITRO HISTAMINE RELEASE FROM ALLERGIC BLOOD

PAUL P. VAN ARSDEL, JR., M.D., AND ELLIOTT MIDDLETON, JR., M.D.,
SEATTLE, WASH.

In a previously reported study we presented data on the quantitative release of histamine from allergic human leukocytes after incubation with varying concentrations of antigen. Histamine release was greatest at certain optimal antigen concentrations (400 to 1,000 P.N.U. per liter whole blood).

Further observations have been made on the blood of pollen-sensitive individuals during specific antigen treatment. Aliquots of blood were incubated with antigen concentrations of from 100 to 40,000 P.N.U. per liter.

In general, release of histamine was lowered at all antigen concentrations during treatment, but this was not always true. An optimal antigen concentration for histamine release was still demonstrable but became progressively higher as treatment continued, reaching levels of 4,000 to 40,000 P.N.U. per liter. Histamine release was suppressed entirely in blood from only one person and actually increased in one other case. These differences did not correlate with clinical improvement, which was marked in all persons studied.

The blocking antibody produced during adequate treatment neutralized 50 to 100 per cent of the added antigen. Little correlation was observed between antigen concentration and degree of neutralization except when concentration exceeded 4,000 P.N.U. per liter, and neutralization was usually slight.

30. EVIDENCE OF ALLERGY IN PATIENTS WITH BRONCHIAL ASTHMA DUE TO CHRONIC CYSTIC FIBROSIS

THOMAS E. VAN METRE, JR., M.D., ROBERT E. COOKE, M.D., EDWARD L. GIBSON, M.D., AND WALTER L. WINKENWERDER, M.D., BALTIMORE, MD.

Previous reports have indicated that bronchial asthma is a frequent manifestation of chronic cystic fibrosis (fibrocystic disease of the pancreas) and probably is secondary to bronchial obstruction produced by the viscid sputum and respiratory infections characteristic of this disease. They have emphasized the importance of distinguishing bronchial asthma caused by this ultimately fatal disorder from bronchial asthma of allergic origin.

During the past three years, we have seen five patients with bronchial asthma who have had evidence of both chronic cystic fibrosis and allergy.

All patients had definite evidence of chronic cystic fibrosis. Two had a family history of this disease, four were malnourished, five had recurrent upper and lower respiratory infections, five had pulmonary scarring, three had clubbed fingers, four had digestive disturbance consequent to deficient pancreatic enzymes, and four were shown to have an elevated concentration of chloride in sweat. The one patient with normal sweat electrolyte concentration had evidence of severe pancreatic insufficiency and chronic pulmonary infection and fibrosis.

All patients had stigmata of allergic disease. Five had a family history of allergy, five had positive skin tests to common environmental dusts or pollens, three had evidence of hay fever, and three had nasal polyps.

The association of allergy and chronic cystic fibrosis described here is believed to represent the chance occurrence of two unrelated disorders in the same patient. Chronic cystic fibrosis appeared to be the major determinant of the asthma observed in these patients. However, allergy seemed to play a contributory role.

31. IN VITRO PRODUCTION OF RABBIT ANTIBODY

J. H. Vaughan, M.D., R. W. Dutton, M. George and A. H. Dutton, M.D., Rochester, N. Y.

The production of antibody by isolated spleen cells taken from animals immunized against crystalline egg albumin has been studied. The spleens of animals killed by a blow to the head were perfused, removed, and minced. The fragments of the spleen were passed through a 60-mesh sieve, providing an isolated cell suspension susceptible to efficient washing procedures. After three washes the cells were suspended in modified Eagles medium containing 15 per cent normal rabbit serum, and aliquots were distributed among 10 ml. beakers. The beakers were covered with sterile gauze, placed in a desiccator jar saturated with water vapor, and gassed with 95 per cent oxygen and 5 per cent CO^2.

Manufacture of new antibody was demonstrated by the appearance of high hemagglutination titer in the supernatant of cells incubated in this fashion for twenty-four hours. No hemagglutinating activity was demonstrable in the supernates of cell controls frozen and thawed at the beginning of the incubation period. The incorporation of C-14 leucine into newly formed antibody could be demonstrated by the appearance of radioactivity in egg albumin anti-egg albumin precipitates formed at the end of the incubation period, the incorporation being linear over a twenty-four-hour period.

At the time of initial incubation the spleen cells had normal morphology for the hyperimmunized state, there being abundant mature and immature plasma cells in addition to large and small lymphocytes, stem cells, and reticuloendothelial cells. During the course of incubation the viability of the cells gradually reduced from 100 per cent to around 65 per cent, as judged by trypan blue staining. Concomitantly, the nuclei of many cells became more pyknotic and the proportion of cells having the superficial characteristics of small lymphocytes became greater.

The applicability of this technique for the demonstration of skin-sensitizing antibody, either of human or of rabbit origin, is under investigation.

32. EXPERIMENTAL DEPLETION OF MONONUCLEAR CELLS IN GUINEA-PIGS

Rudolf E. Wilhelm, M.D., J. Prochazka Fisher, M.D., and Robert A. Cooke, M.D., New York, N. Y.

A rabbit antiserum, directed against guinea-pig mononuclear blood cells, was produced by injections of guinea pig lymph node suspensions to rabbits. This antiserum was modified by incubation with guinea pig erythrocytes and serum to render its effect more specific against mononuclear cells.

This modified rabbit antiserum produced in guinea pigs initial leukopenia, neutrophilia, eosinophilia and a marked drop of mononuclear cells. These effects differed from those caused by normal rabbit serum and were different from the alarm reaction, anaphylaxis, or the nonspecific action of foreign protein injections.

The depletion of mononuclear cells produced by these rabbit anti-guinea pig lymph node serum injections reduced the skin reactivity of DNCB-sensitive guinea pigs to applications of this simple chemical. A similar effect was not observed following injections of normal rabbit serum.

33. CONTINUOUS CORTICOSTEROID THERAPY IN ASTHMA

WALTER L. WINKENWERDER, M.D., AND LESLIE N. GAY, M.D., BALTIMORE, MD.

This study analyzes the results of treating chronic intractable asthma in adults with minimal daily doses of corticosteroids. Such a regimen has been applied for the past eight years to patients exhibiting a favorable response to corticosteroids. Compared to intermittent therapy, the results indicate a smoother clinical course, less physical disability, and correspondingly increased social and economic benefits. The absence of major complications and the restoration of the individual to a useful life indicate that such a regimen under close supervision should be more widely applied.

Obituary

ALBERT VANDER VEER
1878-1959

ALBERT VANDER VEER died on Saturday, Jan. 31, 1959, in the Presbyterian Hospital, Charlotte, North Carolina. During the last two years of his life he had suffered an increasing disability resulting from a slowly progressive heart condition.

Dr. Vander Veer was born in Albany, the son of one of New York State's most illustrious surgeons. His two brothers, who predeceased him, were likewise prominent surgeons in Albany.

Albert Vander Veer graduated from Yale University in 1900 and received his M.D. degree from the College of Physicians and Surgeons, Columbia University, in 1904. Following this he had a two-year internship at Roosevelt Hospital and entered the practice of internal medicine in New York City.

Dr. Vander Veer's professional career was a noteworthy one. He soon became interested in the new field of hypersensitiveness with his friend and associate, Robert A. Cooke. Their professional relationship lasted until Dr. Vander Veer retired in 1947. In 1916 they published one of their first studies on human hypersensitivity, a new branch of medicine which later came to be known as allergy.

In this field Dr. Vander Veer was an eminent pioneer and was one of the founders of the clinic at New York Hospital—the first one established for the study of causes and treatment of allergic diseases of man. Dr. Vander Veer's contributions to this work were outstanding, and during his active professional career his work was centered along these lines.

Dr. Vander Veer was also one of the founders of the original Society for the Study of Asthma and Allied Diseases, and he was president of that society in 1932. He was also a member of the county, state, and national medical societies and a Fellow of the Academy of Medicine and the American Academy of Allergy.

He retained his connection with the Allergy Clinic when it moved to Roosevelt Hospital in 1932. He was a member of the Medical Board of that hospital and served as consultant in allergy as long as he remained in active practice.

The only interruption in this long service to the hospital was during World War I, when he served with distinction as a major in the Army Medical Corps in France. Later he was commissioned a lieutenant colonel in the Army Medical Reserve Corps.

He was a member of the American Legion, Sons of the Revolution, Holland Society, and the University Club and Yale Club of New York.

A man of sincere religious convictions, he was a member of the Fifth Avenue Presbyterian Church and served for many years as an elder in the governing body of that church.

His first wife, Sylvia de Minos Vander Veer, died in 1944. He is survived by his second wife, Margaret Clark Vander Veer, to whom he was married in 1951, and by his two daughters, Mrs. Charles Colmore, Jr., and Mrs. H. G. Leland, Jr., and their six children.

It is one thing to thus enumerate some of the major interests and activities of a long and fruitful life. I find it much more difficult to express adequately in words the tribute that I would like to pay to the memory of the man with whom I was so intimately associated for more than four decades. Albert Vander Veer was a man of rare and fine qualities and noble instincts. He combined a delicious and subtle sense of humor with a genuineness and kindliness that made him beloved by all who had the privilege of contact with him. Society will long be indebted to him for the influence he exerted in his quiet, gentle manner.

R. A. C.

Book Review

Third International Congress of Allergology. B. N. Halpern and A. Holtzer (editors). Paris, 1958, Ernest Flammarion. 940 pages.

This volume includes papers on a wide variety of subjects within the field of allergy which were presented at the Third International Congress of Allergology in October, 1958, in Paris. The book is composed of seven symposia, the first of which deals with various aspects of emphysema and asthma, with special emphasis on the pathologic physiology of obstructive pulmonary disease. The second is a discussion of the nature of antibodies, and the third deals with recent advances on the treatment of allergic diseases. It is interesting to note that the third symposium contains the only paper in the volume devoted to the corticosteroids and their influence on allergic states. The subject of histamine and histamine liberators is well covered in the fourth symposium, and autoimmune disease is taken up in the fifth. The sixth deals with atopic dermatitis. The seventh and last symposium discusses the sociologic impact of allergic diseases in various parts of the world. This will undoubtedly prove to be a very valuable contribution, and it is certainly an original one. Contributors from more than twenty countries discuss the problems peculiar to their region or those aspects of the field which have been of greatest interest. Many of the papers are well documented with references to the pertinent literature.

The reader who restricts himself to perusal of the English summaries of those papers in French, German, and Spanish should be aware that there are a number of errors in translation, a few of which might be either confusing or misleading. A case in point is on page 681, where the English version states: "Cortico-therapy is now the leading treatment for cutaneous cures." No word that can be translated as "cures" appears in the original French version.

Review of a volume which includes so much diversity in content and approach can hardly be anything more than a cursory cataloguing of its contents. Anyone concerned with the field of allergy will find much of interest in these seven symposia, and since more than half the contributions in the first six are in English, the volume should have a wide appeal in English-speaking countries.